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16. ABSTRACT

This study was conducted to identify possible adverse effects on human health from slow-rate (sprinkler) land application of wastewater which contained pathogens. An epidemiological study of 478 area residents and farm workers was maintained during the first 20 months of sprinkler operation and during the 20-month period immediately preceding initiation of wastewater irrigation. Blood samples were analyzed for antibody titers to 23 viruses, Legionella and Entamoeba histolytica. Fecal specimens were collected to isolate pathogens; electron microscopic examination was performed to detect virus-like particles, and tuberculin skin tests were administered. Weekly illness information was provided by participants. Microorganisms were measured in the wastewater, aerosols and drinking water. An index of relative cumulative exposure of each participant to the wastewater aerosol was calculated. High levels of bacteria and enteric viruses were present in the wastewater obtained via pipeline from the treatment plant. Enteroviruses were found in the wastewater aerosols. Disease surveillance did not disclose any obvious connection between acute illness and aerosol exposure but three different statistical approaches indicated that the rate of viral infections was slightly higher among participants who had a high degree of aerosol exposure. However, it could not be determined whether aerosol exposure or alternative explanations were the actual risk factor(s) in these infections. The association of viral infections with aerosol exposure showed a dose effect.

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THE LUBBOCK LAND TREATMENT SYSTEM RESEARCH AND DEMONSTRATION PROJECT

VOLUME IV

Lubbock Infection Surveillance Study (LISS)

by

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NOTICE

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, North Carolina, and Cincinnati, Ohio, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides and nonionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report describes a 5-year prospective epidemiological study to investigate potential infectious disease effects from sprinkler application of wastewater to land. With a better understanding of health effects, measures can be developed to reduce exposure to harmful materials.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

PREFACE

The LCC Institute of Water Research (LCCIWR), Lubbock, Texas, conducted a 5-year (1979-1983) research and demonstration program entitled the Lubbock Land Treatment Project to expand and study Lubbock's municipal wastewater land treatment system. A pipeline, storage reservoirs, distribution system, and spray irrigation equipment were installed at the Hancock farm site, located about 15 miles southeast of the sewage treatment plant and the edge of Lubbock. The research programs of the Lubbock Land Treatment Project included ground water recovery studies at a farm practicing land application of wastewater for over 40 years (the Gray site), a health effects study at the Hancock site, and impact studies on crops, soil and ground water.

As part of the Lubbock Land Treatment Project, a 5-year health effects study (Lubbock Infection Surveillance Study, LISS) was performed to investigate potential infectious disease effects from sprinkler application of wastewater to land. The health effects study (Volume IV) is the subject of this report. The other volumes in this series are:

Volume I.	Demonstration-Hydrogeologic Study
Volume II.	Percolate Investigation in the Root Zone
Volume III.	Intensive Agricultural Research Studies
Volume V.	Executive Summary

ABSTRACT

The Lubbock Infection Surveillance Study (LISS) was conducted to monitor infections and acute illness in the primarily rural community surrounding the Lubbock Land Treatment (Demonstration) System (LLTS) at the Hancock farm near Wilson, Texas. The LISS objective was to identify possible adverse effects on human health from slow-rate (sprinkler) land application of wastewater which contained potentially pathogenic microorganisms.

An epidemiological analytic cohort study of 478 area residents and Hancock farm workers was maintained during the first 20 months of operation of the LLTS (February 1982-October 1983) and during the 20-month period immediately preceding LLTS operation (June 1980-January 1982). Blood samples collected semiannually were analyzed for antibody titers to 14 enteroviruses, 3 adenoviruses, 2 reoviruses, rotavirus, Norwalk virus, hepatitis A, *Legionella*, *Entamoeba histolytica*, and influenza A. Routine fecal specimens were collected regularly to isolate enteric viruses and overt and opportunistic bacterial pathogens. Electron microscopic examination was performed to detect a variety of other virus-like particles. Tuberculin skin tests were administered annually to detect non-tuberculosis mycobacterial infections. Illness information was provided by study participants on a weekly basis. Concentrations of microorganisms also were measured in the wastewater, wastewater aerosol, and drinking water. Dispersion modeling, participant activity diaries, and a weekly log of extensive wastewater contact were used to calculate an aerosol exposure index of relative cumulative exposure of each participant to the wastewater aerosol within each of the four major irrigation seasons.

Very high levels of bacteria and enteric viruses were present in the sprayed wastewater obtained via pipeline directly from the Lubbock sewage treatment plant. Enteroviruses were consistently found in the wastewater aerosol in 1982.

Participants in the high and low exposure groups were generally well balanced with regard to age, gender, previous titer, and time spent in Lubbock. However, aerosol exposure was largely confounded with patronage of a local restaurant and use of evaporative cooler air conditioners.

Disease surveillance did not disclose any obvious connection between the self-reporting of acute illness and degree of aerosol exposure.

Whenever a sufficient number of infections was observed during an irrigation season, this infection episode was analyzed by four different methods: confirmatory statistical analysis, exploratory logistic regression analysis, confidence intervals of incidence density ratios, and risk ratio scoring. The association of infection status with wastewater aerosol exposure and other relevant factors was investigated.

Comparison of crude seroconversion incidence densities indicated that some excess risk of viral infection (risk ratio of 1.5 to 1.8) appeared to be associated with level of aerosol exposure. A symmetric risk ratio scoring approach provided evidence of a dose-related stable association (p=0.002) between the infection events in the observed episodes of infection and aerosol exposure. More than the expected number of statistically significant associations of the presence of infection with wastewater aerosol exposure were found in the confirmatory analysis of independent infection episodes using Fisher's exact test. Thus, three different statistical approaches provided similar evidence that the rate of viral infections was slightly higher among members of the study population who had a high degree of aerosol exposure.

In the episode of poliovirus 1 seroconversions in spring 1982, some of the infections were probably caused by wastewater aerosol exposure because a strong association existed and no alternative explanation could be identified. Three distinct risk factors (poliovirus immunization in spring 1982, low polio 1 antibody titer in January 1982, and a high degree of aerosol exposure) were independently associated with the poliovirus 1 seroconversions and each appears to have been responsible for some of the poliovirus 1 infections. Weak evidence of association was found between aerosol exposure and infection by other enteric viruses (specific coxsackie B viruses and echoviruses) which were simultaneously recovered from the wastewater during the summer irrigation season of 1982. However, it could not be determined whether aerosol exposure or identified alternative explanations were the actual risk factor(s) in these enteric viral infections. The association of viral infections with aerosol exposure shows a dose effect, since the study population was exposed to more enteroviruses via the wastewater aerosol in 1982 than in 1983.

The LISS was conducted by Southwest Research Institute (SwRI), the University of Illinois (UI), the University of Texas at San Antonio (UTSA) and the University of Texas at Austin (UTA). This report was submitted in fulfillment of CR 807501 and S806204 by SwRI under primary sponsorship of the U.S. Environmental Protection Agency. This report covers field activities performed from May 1, 1980 to October 31, 1983; work was completed as of June 30, 1985.

CONTENTS

Page

Foreword.......... iii
Preface.......... iv
Abstract.......... v
Figures.......... xiii
Tables.......... xiv
Abbreviations.......... xx
Acknowledgement.......... xxii

1. Introduction.......... 1
   A. Background.......... 1
      Land application and potential infectious disease hazards.......... 1
      Recent literature.......... 2
      The Lubbock Land Treatment System (LLTS) expansion.......... 3
      The Lubbock Infection Surveillance Study (LISS).......... 3
   B. Study Objective.......... 5
   C. Study Design.......... 5
   D. Study Organization.......... 10
2. Conclusions.......... 13
3. Recommendations.......... 17
4. Methods and Materials.......... 20
   A. Study Site.......... 20
      Description of study area.......... 20
         General climatology.......... 20
         City of Wilson.......... 21
         Rural area.......... 21
      Lubbock sewage treatment plants.......... 22
      Lubbock land treatment system.......... 23
      System design and operation in relation to EPA design criteria and recommendations.......... 24
      Periods of irrigation.......... 27
   B. Study Population.......... 28
      Sampling.......... 28
      Health interview and recruitment.......... 31
      Serosurvey.......... 32
      Fecal specimens.......... 33
      Illness and exposure monitoring.......... 34
      Illness specimens.......... 36
      Activity diaries.......... 37
      Tuberculin skin testing.......... 37
      Poliovirus immunization.......... 38
      Restaurant patronage survey.......... 39
   C. Exposure Estimation.......... 40
      Aerosol exposure index (AEI).......... 40
      Additional exposure measures.......... 44

CONTENTS (CONT'D)

Page

D. Environmental Sampling.. 46
  Wastewater.. 46
  Wastewater aerosol.. 48
    Background runs--1980 baseline year....................... 48
    Wastewater aerosol monitoring--1982 irrigation year....... 51
      Microorganism runs...................................... 51
      Quality assurance runs.................................. 54
      Enterovirus runs.. 54
      Dye runs.. 55
      Particle size runs...................................... 56
      Dust storm runs... 56
  Calculation of microorganism density in air................. 56
  Flies... 58
  Drinking water.. 59
  Meteorological data... 60
    Background aerosol runs................................... 60
    General climatology....................................... 60
    Meteorological measurements during aerosol runs........... 62
E. Laboratory Analysis of Clinical Specimens..................... 62
  Serology.. 62
    Enteroviruses... 62
    Adenoviruses.. 65
    Hepatitis A... 65
    Influenza... 65
    Legionella bacilli...................................... 65
    Norwalk virus... 66
    Entamoeba _histolytica_................................. 66
    Reoviruses.. 66
    Rotavirus... 66
  Clinical bacteriology....................................... 67
  Clinical virology... 71
  Electron microscopy of fecal specimens...................... 73
F. Laboratory Analysis of Environmental Samples.................. 74
  Wastewater samples.. 74
    Microbiological screens................................... 74
    Routine wastewater samples................................ 82
    Enterovirus identification samples........................ 82
    Limited bacterial screen samples.......................... 82
    Legionella samples...................................... 83
  Aerosol samples... 84
  Fly samples... 85
  Drinking water samples...................................... 85
G. Infection Events and Episodes................................. 87
  Bacterial infection event................................... 87
  Viral infection event....................................... 89
  Serological infection event................................. 89
  Identification of infection episodes........................ 89

CONTENTS (CONT'D)

Page

H. Data Management ... 97
  Data processing and verification ... 97
  Data base structure and use ... 98
I. Quality Assurance ... 100
  Health data and specimens ... 100
  Aerosol measurement precision ... 100
  Laboratory analysis ... 102
    Enterovirus serology ... 102
    Hepatitis A serology ... 104
    Clinical bacteriology ... 104
    Clinical virology ... 105
    Electron microscopy ... 106
    Environmental samples ... 106
  Data management ... 108
  Archiving of clinical specimens ... 108
J. Statistical Methods ... 108
  Preliminary analysis ... 110
  Confirmatory analysis ... 111
    Testing procedure ... 111
    Stratification ... 112
  Exploratory analysis ... 112
  Analysis of risk ratio (RR) scores ... 113
  Analysis of incidence density ratios (IDR) using test-based confidence intervals ... 116
  Other analyses of apparent association of infections with exposure ... 119
K. Interpretation of Statistical Results ... 120
5. Results ... 125
A. Microorganism Levels in Wastewater ... 125
  24-Hour composite samples--overview ... 125
  24-Hour composite samples--bacterial pathogens ... 127
  24-Hour composite samples--human enteric viruses ... 131
  24-Hour composite samples--geometric mean data ... 134
  30-Minute composite samples ... 134
B. Microorganism Levels in Air ... 138
  Aerosolization efficiency ... 138
  Size of viable particles in the wastewater aerosol ... 141
  Background microorganism densities in ambient air ... 143
  Microorganism densities in downwind air from microorganism runs ... 145
  Enterovirus densities in downwind air from virus runs ... 150
  Microorganism exposure via the wastewater aerosol ... 153
  Estimates of aerosol exposure index (AEI) and other participant exposure measures ... 156

CONTENTS (CONT'D)

Page

C. Other Investigated Sources of Microorganisms 162
  Microorganism levels on flies 162
  Microorganism levels in drinking water 165
  Eating food prepared at local restaurants 171
    Restaurant A 172
    Restaurant B 173
    Discussion 173
D. Description of Study Population 174
  Questionnaire data 174
  Population demographics 175
    Effect of self-selection on LISS population characteristics 179
    Characteristics of subpopulations 179
    Characteristics of donor groups 181
    Exposure categories based on aerosol exposure indices 182
  Samples provided by study population during the health watch 183
E. Patterns in Self-Reported Illness 183
  Baseline 188
  Irrigation-1982 197
  Irrigation-1983 199
  Discussion 200
F. Surveillance via Illness and Requested Specimens 201
  Illness investigations 201
  Group A streptococci 209
  Enteric Gram-negative bacteria (EGNB) 210
  Viruses 219
G. Clinical Bacteriology of Routine Fecal Specimens 222
  Summary data 222
  Bacterial infection events 226
  Infections by overt pathogens 226
  Klebsiella infections 227
  Infections by non-*Klebsiella* Category 2 bacteria (other opportunistic bacteria) 230
  Infections by bacteria prominent in wastewater 230
H. Clinical Virology of Routine Fecal Specimens 233
I. Serologic Data and Seroconversion Rates 243
  Antibody prevalence 243
  Incidence densities for serologic agents 244
  Identified serologic infection episodes 254
J. Other Infections: Mycobacteria, Parasites, and Coronavirus-like particles 255
  Nontuberculosis mycobacterial (NTM) infections from tuberculin skin testing 255
  Parasite infestation 257
  Electron microscopy (EM) of routine fecal specimens 259

CONTENTS (CONT'D)

Page

K. Observed Episodes of Infection 265
  Infection incidence rates of infection episodes 265
  Evaluation of association of infections with aerosol exposure via risk ratio scores 279
L. Statistical Analysis 282
  Preliminary analysis 282
  Confirmatory analysis 295
  Exploratory logistic regression analysis 302
    Analysis 1: basic analysis 302
    Analysis 2: investigate possible restaurant etiology 317
    Analysis 3: exclude AEI to investigate alternative explanations 317
    Analysis 4: investigate route of wastewater exposure 317
  Evaluation of the effect of ignoring multiple infection events on the statistical analysis results 321
M. Evidence of association of specific infection episodes with wastewater aerosol exposure 325
6. Discussion 339
  A. Prior wastewater aerosol health effect studies 339
  B. Summary of LISS findings 342
    Findings from wastewater and aerosol data 342
    Findings from self-reported illness data 342
    Findings from nonepisode occurrences of infections 344
    Findings from seroconversion incidence density 346
    Findings from risk ratio scoring of infection episodes 347
    Findings from confirmatory statistical analysis of infection episodes 348
    Findings from exploratory statistical analysis of infection episodes 349
    Evidence of association of specific infection episodes with wastewater aerosol exposure 349
  C. Comparison of Findings to the Literature 351
    Self-reported illness 351
    Bacterial agent episodes 352
    Viral agent episodes 356
  D. Significance of Findings 359
References 362

Appendixes

A. Supplemental Figures and Tables for Section 4 (Methods and Materials) 374
B. Initial Personal Interview Questionnaire 422
C. Personal Questionnaire Update in February 1982 436
D. Personal Questionnaire Update in October 1983 444
E. Informed and Parental Consent Forms 455
F. Household Health Diary Booklet (1980) 461
G. Health Diary Forms and Weekly Illness Surveillance Summary (1982 and 1983) 465

CONTENTS (CONT'D)

Page

H. Activity Diaries and Maps.. 471
I. Wilson Eating Establishment Survey Form........................... 480
J. Procedure for Wastewater Sample Collection, Lubbock Southeast Water Reclamation Plant.. 482
K. Procedure for Wastewater Sample Collection, Wilson Imhoff Tank Effluent.. 487
L. Description of Litton Model M High Volume Aerosol Sampler......... 490
M. Decontamination Procedure for Model M Samplers.................... 496
N. Collection Efficiency of Litton Model M Large Volume Samplers..... 498
O. Enterovirus Serology Quality Control: Titer Reproducibility (TR) from Replicate Testing.. 508
P. Supplemental Tables for Section 5 (Results)....................... 524

Glossary.. 611

FIGURES

Number Page

1 Wastewater irrigation system.. 4
2 LISS study design: timeframe of monitoring in relation to major periods of irrigation.. 6
3 Principal investigators and functional areas........................ 11
4 Hancock farm irrigation system....................................... 25
5 Wastewater sprinkler irrigation at Hancock farm during LISS...... 29
6 Sampling zones comprising the study area........................... 30
7 Relation of activity diary collection weeks to major periods of irrigation.. 43
8 Sampler locations for background runs............................. 49
9 Typical sampler configuration for microorganism run.............. 52
10 Typical sampler configuration for quality assurance and enterovirus runs.. 52
11 Typical sampler configuration for dye run.......................... 53
12 Typical sampler configuration for particle size run.............. 53
13 Drinking water sampling locations.................................. 61
14 Isolation and identification of selected organisms from feces.... 68
15 Isolation and identification of organisms from throat swabs...... 69
16 Viral isolation from clinical specimens............................ 72
17 Isolation of gram-negative enteric bacteria from wastewater...... 79
18 Analyses of insect vectors.. 86
19 Incidence density rates by exposure level for total acute illness by month--1980.. 191
20 Incidence density rates by exposure level for total acute illness by month--1981.. 191
21 Incidence density rates by exposure level for total acute illness by month--1982.. 192
22 Incidence density rates by exposure level for total acute illness by month--1983.. 192
23 Incidence density rates by exposure level for respiratory illness by month--1980.. 193
24 Incidence density rates by exposure level for respiratory illness by month--1981.. 193
25 Incidence density rates by exposure level for respiratory illness by month--1982.. 194
26 Incidence density rates by exposure level for respiratory illness by month--1983.. 194
27 Virus particles observed by EM in illness stool specimens........ 221
28 Coronavirus-like particles observed by EM in routine stool specimens.. 261

TABLES

Number Page

1 Suggested prevalence of antibody and seasonal occurrence of infection for agents potentially present in wastewater........ 7
2 Frequency of measurement in wastewater of interpretable infectious agents monitored in the health watch............... 9
3 Principal participating personnel and areas of activity......... 12
4 Major irrigation periods at Hancock farm during LISS surveillance.. 27
5 Minor irrigation periods at Hancock farm during LISS surveillance.. 28
6 Comparison of sentinel population to study population in October 1982.. 36
7 Summary of participant poliovirus protection status............. 39
8 Epidemiologic characteristics of candidate agents for serologic testing...................................... 63
9 Agents and sera selected for use in serologic testing........... 64
10 Bacterial infection criteria.................................... 88
11 Number of cases required for rejection of $P_1=P_2$ in favor of $P_1<P_2$ if all cases occur in the smaller group and none occur in the larger group.. 90
12 Infection episode criteria...................................... 91
13 Infection episode dependent variable name key.................. 93
14 Agents comprising clinical WWI episode by season: wastewater isolates recovered in routine fecal specimens during same irrigation period.. 94
15 Classification criteria for jointly independent groups of infection episodes.. 97
16 Structure of LISS data base..................................... 99
17 Risk ratio score criteria....................................... 115
18 Definitions for agent groupings in serologic data analysis...... 118
19 Criteria for strength and consistency of apparent association of infections with wastewater aerosol exposure in infection episodes... 122
20 Criteria for judging quality of wastewater evidence for each microorganism.. 123
21 Quality of wastewater applied by sprinkler irrigation........... 126
22 Bacterial screens--Lubbock, Texas............................... 128
23 Bacterial screen--Hancock reservoir............................. 129
24 Species of Legionella detected in wastewater samples by direct fluorescent antibody staining of the original samples or tissues from guinea pigs innoculated with those samples....... 130
25 Viruses isolated from Lubbock pipeline effluent during 1982..... 132
26 Viruses isolated from Lubbock pipeline effluent during 1983..... 133
27 Viruses isolated from Hancock farm reservoirs during 1983....... 133

TABLES (CONT'D)

Number Page

28 Geometric mean of microorganism concentrations in Lubbock wastewater.......... 135
29 Geometric mean of microorganism concentrations in Hancock reservoir wastewater.......... 137
30 Calculated concentrations and corresponding aerosolization efficiency point estimates for each sampler during each dye run.......... 140
31 Summary of aerosolization efficiency of the Hancock farm irrigation system in 1982.......... 141
32 Standard plate count density of viable particles in air by distance and particle size.......... 142
33 Geometric mean background densities in ambient air sampled on background runs.......... 144
34 Estimated densities sampled on microorganism and virus aerosol runs.......... 146
35 Confirmation of spray irrigation of pipeline wastewater as a significant source of microorganisms in downwind air: paired downwind versus upwind densities.......... 147
36 Microorganism densities in air at Hancock farm compared to other wastewater treatment facilities.......... 149
37 Viruses recovered from aerosol samples during virus runs.......... 150
38 Sampled enterovirus densities on virus runs.......... 151
39 Identification of viral isolates recovered during virus runs.......... 152
40 Estimated microorganism densities in air downwind of irrigation in 1982 relative to ambient background levels near homes and in fields.......... 154
41 Significant elevation of microorganism density in air downwind of spray irrigation with pipeline wastewater relative to ambient background outside participant homes.......... 155
42 Relative aerosol exposure measure to sprayed microorganisms bv irrigation period and downwind distance.......... 157
43 Distribution of participant aerosol exposure index by irrigation period.......... 158
44 Relative contribution of irrigation seasons to total enteroviruses sprayed for 1982, 1983 and entire irrigation period... 160
45 Bacterial isolates from flies.......... 164
46 Microorganism densities in drinking water in the study area by well location and sampling date.......... 166
47 Precipitation by month in the study area.......... 171
48 Frequency distributions of patronage of major food preparation facilities in Wilson by 117 fecal and illness specimen donors during irrigation periods.......... 172
49 Variables used in demographic analysis.......... 176
50 Comparison of characteristics: study participants versus nonparticipants.......... 177

TABLES (CONT'D)

Number Page

51 Comparison of charactertistics: participants who remained in the study versus participants who dropped out.............. 178
52 Number of samples collected from health watch activities........ 184
53 Monthly intervals for self-reported illness data by date and DCP 187
54 Monthly incidence density of self-reported illnesses by type of illness and exposure level.................................. 189
55 Monthly incidence density of self-reported illnesses by type of illness and exposure group.................................. 190
56 Monthly prevalance density of self-reported illnesses by type of illness and exposure level.................................. 195
57 Monthly prevalence density of self-reported illnesses by type of illness and exposure group............................ 196
58 Bacteriology throat swab series for donors with moderate or heavy levels of enteric Gram-negative bacteria in an illness throat swab.. 204
59 Occurrence of abnormal throat flora in acute and convalescent illness throat swabs... 208
60 Microorganisms found in the oropharynx.......................... 210
61 Investigation of various donor exposure variables for association with enteric Gram-negative bacteria in illness throat swabs in summer 1982.. 212
62 Clinical bacteriology results from requested throat swab surveys of healthy participants in September 1982 and June 1983....... 213
63 Investigation of various donor exposure variables for association with enteric Gram-negative bacteria in requested throat swab survey of healthy donors in September 1982............... 214
64 Occurrence of abnormal levels of flora in acute and convalescent illness fecal specimens.......................... 217
65 Age-specific distribution of abnormal levels of flora in illness fecal specimens...................................... 218
66 Identification and comparison of viral isolates by cell culture and virus-like particles by EM in illness fecal specimens..... 220
67 Organisms isolated from routine fecal specimens during 1980 and 1981.. 223
68 Organisms isolated from routine fecal specimens during 1982..... 224
69 Organisms isolated from routine fecal specimens during 1983..... 225
70 Infections by overt enteric bacterial pathogens................ 226
71 Prevalence of bacterial infections by collection month.......... 228
72 Exploratory analysis of the association of individual characteristics with infection prevalance..................... 229
73 Association of level of Klebsiella growth in routine fecal specimens with the incidence of self-reported illness in the prior, concurrent and subsequent biweekly reporting periods.......... 231
74 Episodes of bacterial infection detected from routine fecal specimens during irrigation seasons.......................... 232

TABLES (CONT'D)

Number Page

75 Association of level of growth of prominent wastewater bacteria in routine fecal specimens with the incidence of self-reported illness in the prior, concurrent and subsequent biweekly reporting periods 234
76 Prevalence and identification of viral isolates recovered from routine fecal specimens by collection month 235
77 Age-specific annual recovery of viral isolates from routine fecal specimens 236
78 Viral isolates recovered from donors of routine fecal specimens during baseline monitoring 238
79 Viral isolates recovered from donors of routine fecal specimens in 1982 239
80 Viral isolates recovered from donors of routine fecal specimens in 1983 240
81 Association of viral isolates in routine fecal specimens with the incidence of self-reported illness in the prior, concurrent and subsequent biweekly reporting periods 241
82 Episodes of infection to viruses detected from routine fecal specimens during irrigation seasons 242
83 Effect of immunization on participant poliovirus titers by agent and vaccine type 244
84 Comparison of baseline and irrigation incidence density rates by wastewater aerosol exposure level and agent 246
85 Comparison of baseline and irrigation infection incidence density rates by wastewater aerosol exposure group and agent .. 248
86 Comparison of baseline and irrigation incidence density rates by wastewater aerosol exposure level and agent grouping 250
87 Comparison of baseline and irrigation incidence density rates by wastewater aerosol exposure group and agent grouping 251
88 Infection incidence density rates for wastewater aerosol exposure levels by agent grouping and time interval 252
89 Prevalence of mycobacteria response from initial Mantoux tuberculin skin test results 255
90 Incidence of mycobacteria infections from tuberculin testing of study population 256
91 Ova and parasite survey of LISS population 258
92 Aerosol exposure comparison of *Giardia*-positive and *Giardia*-negative fecal donors in ova and parasite survey 259
93 Occurrence of coronavirus-like particles in routine fecal specimens examined by electron microscopy 262
94 Electron microscopy results for routine fecal specimen series of donors positive for coronavirus-like particles 263
95 Age-specific prevalence of coronavirus-like particles detected by electron microscopy in routine fecal specimens 264
96 Average aerosol exposure comparison of coronavirus-like infected donors versus noninfected donors during irrigation seasons in 1982 264

TABLES (CONT'D)

Number Page

97 Clinical infection episodes.................................... 266
98 Serologic infection episodes to single agents.................. 267
99 Serologic infection episodes to groups of agents............... 270
100 Infection incidence rates by exposure groups and levels and risk ratio score of infection episodes classified as exposure situations.. 271
101 Infection incidence rates by exposure groups and levels and risk ratio score of infection episodes classifed as control situations.. 276
102 Significance of frequency distributions of risk ratio scores by group of jointly independent infection episodes............ 280
103 Comparison of exposure groups with respect to household characteristics by baseline and irrigation season--blood donors..... 283
104 Comparison of exposure groups with respect to household characteristics by baseline and irrigation year--blood donors....... 285
105 Comparison of exposure groups with respect to household characteristics--fecal donors................................ 286
106 Comparison of exposure groups with respect to individual characteristics--blood donors................................ 288
107 Comparison of exposure groups with respect to individual characteristics--fecal donors................................ 290
108 Comparison of exposure groups with respect to previous titer to serologic agents.. 292
109 Comparison of exposure groups with respect to frequency of eating food prepared at restaurants A and B--fecal donors..... 293
110 Comparison of incidence of bacterial infections in low and high exposure groups.. 296
111 Comparison of incidence rates of viral infections in low and high exposure groups.. 297
112 Comparison of incidence of serologic infections in low and high exposure groups.. 298
113 Comparison of incidence of polio infections in low and high exposure groups stratified by immunization status............ 299
114 Rate of positive associations detected by the statistical confirmatory analysis at significance level 0.05 in independent infection episodes.................................. 300
115 Previous titer and response variables for logistic regression analysis.. 303
116 Predictor variables for logistic regressions................... 304
117 Predictor variables used in logistic regression analysis........ 306
118 Logistic regression results for baseline infection episodes..... 308
119 Logistic regression results for spring 1982 infection episodes.. 309
120 Logistic regression results for summer 1982 infection episodes.. 310
121 Logistic regression results for spring 1983 infection episodes.. 311
122 Logistic regression results for summer 1983 infection episodes.. 312
123 Logistic regression results for 1982 infection episodes......... 313

TABLES (CONT'D)

Number Page

124 Logistic regression results for 1983 infection episodes......... 314
125 Results of rerun of Analysis 1--investigate infection episodes with fewer observations deleted.............................. 315
126 Results of Analysis 2--investigate possible restaurant etiology. 318
127 Results of Analysis 3--exclude AEI to investigate alternative explanations.. 320
128 Results of Analysis 4--investigate route of wastewater exposure. 322
129 Effect of multiple infection events on confirmatory analysis results... 323
130 Effect of multiple infection events on exploratory logistic regression analysis results................................. 324
131 Summary of findings for control infection episodes: evidence regarding spurious association of infections with wastewater aerosol exposure.. 327
132 Summary of findings for exposure infection episodes: evidence regarding association of infections with wastewater aerosol exposure... 329
133 Summary of evidence for infection episodes showing strong association of infections with wastewater aerosol exposure.... 335
134 Summary of findings pertaining to possible association with wastewater irrigation for occurrences of infections not classified as infection episodes............................. 345

ABBREVIATIONS

AEI -- aerosol exposure index
AGI -- all-glass impinger
API -- Analytab Products, Incorporated
ATCC -- American Type Culture Collection
BGM -- buffalo green monkey kidney cells
BHI -- brain-heart infusion
BOD$_5$ -- 5-day biochemical oxygen demand
CA -- confirmatory analysis
CAL -- cellobiose arginine lysine agar
CDAS -- cassette data acquisition system (Climatronics Corporation)
cfu -- colony-forming unit
CI -- confidence interval
CMH -- Cochran-Mantel-Haenszel (χ^2 statistics)
CPE -- cytopathic effect
CVLP -- coronavirus-like particles
CYE -- charcoal-yeast extract
DCP -- data collection period
DE -- diatomaceous earth
DFA -- direct fluorescent antibody
DRCM -- differential reinforced Clostridia medium
EGNB -- enteric Gram-negative bacteria
EI -- exposure index
ELISA -- enzyme-linked immunosorbent assay
ELR -- exploratory logistic regression
EM -- electron microscope
EMB -- eosin methylene blue
EWS -- electronic weather station (Climatronics Corporation)
FA -- fluorescent antibody
FHRSEL -- level of farm exposure hours
FHRSEM -- index of farm exposure hours
FITC -- fluorescein isothiocyanate
GI -- gastrointestinal
GMT -- geometric mean titer
GN -- Gram-negative
HAEI -- household aerosol exposure index
HAV -- hepatitis A virus
HI -- hemagglutination-inhibition
HID$_{50}$ -- human infective dose, 50th percentile
ICU -- intensive care unit
ID -- participant identification number
ID -- incidence density
IDR -- incidence density ratio
IFA -- indirect fluorescent antibody
IgG -- immunoglobulin G
IHA -- indirect hemagglutination
IPV -- inactivated polio vaccine (Salk)

ABBREVIATIONS (CONT'D)

IR	-- incidence rate
ISCO	-- Instrumentation Specialties Company
KEC	-- Klebsiella, Enterobacter and Citrobacter
LIA	-- lysine-iron agar
LISS	-- Lubbock Infection Surveillance Study
LLTS	-- Lubbock Land Treatment System
LTFP	-- Lubbock Trickling Filter Plant 2
LVS, LVAS	-- large volume air sampler
Mac, MAC	-- MacConkey agar
MF	-- membrane filtration
MIO	-- motility-indole-ornithine
MPN	-- most probable number
MRI	-- Meteorology Research, Incorporated
NS-PT	-- 0.85% sodium chloride with 25 µg/mL potassium tellurite
NTM	-- non-tuberculosis mycobacteria
O-P	-- ova-parasite
OPV	-- oral polio vaccine (Sabin)
PBS	-- phosphate buffered saline
PBS-Man	-- phosphate buffered saline with 1% mannitol
pfu	-- plaque-forming unit
PPD-S	-- purified protein derivative-stabilized (tuberculin test)
PTA	-- phosphotungstic acid
QA	-- quality assurance
RAEM	-- relative aerosol exposure measure
RD	-- rhabdomyosarcoma
RIA	-- radioimmunoassay
SDA	-- Sabouraud dextrose agar
RR	-- risk ratio
SeWRP	-- Southeast Water Reclamation Plant
SIR	-- Scientific Information Retrieval
SS	-- Samonella-Shigella
$TCID_{50}$	-- tissue culture infective dose, 50th percentile
TKN	-- Total Kjeldahl nitrogen
TLUBOCK	-- time spent in Lubbock
TOC	-- total organic carbon
TPB	-- tryptose-phosphate broth
TR	-- titer reproducibility
TSA	-- trypticase soy agar
TSI	-- triple sugar iron
TSS	-- total suspended solids
TU	-- tuberculin unit
TVSS	-- total volatile suspended solids
URI	-- upper respiratory illness
WIT	-- Wilson Imhoff tank
XAEREL	-- level of extensive aerosol exposure
XAEREM	-- index of extensive aerosol exposure
XDIREL	-- level of extensive direct wastewater contact
XDIREM	-- index of extensive direct wastewater contact
XLD	-- xylose-lysine-deoxycholate
ZM	-- zero-max

ACKNOWLEDGEMENT

We would like to acknowledge the patience, understanding and cooperation of the study participants, especially the 306 participants who stayed with us until October 1983. Their willingness to provide necessary information and to comply with our numerous requests for samples is deeply appreciated. Without their commitment, the study would not have been possible. We are thankful that they allowed us to intrude into their private lives and are grateful that we had an opportunity to get to know these very special people.

A special thanks to the City of Wilson officials, especially City Secretary Naoma (Shorty) Moore, for the help that they provided to the LISS staff. The city council allowed us to use city facilities to store project supplies as well as to collect and process blood and fecal specimens. On occasion, these less than aesthetically pleasing activities disrupted city business, and we are grateful for the humor and the patience exhibited by the city staff during those trying times.

We also acknowledge the vital contribution of the many technicians and clerical personnel who assisted us in this study. Their competence and special skills are appreciated. This list includes the technicians from SwRI who were involved in the wastewater aerosol sampling and fecal collection, technicians at UTSA and UT-Austin who analyzed clinical and environmental samples, technicians at UI and UTSA who performed serologic analyses, phlebotomists from the Lubbock area who drew all of the blood samples, public health nurses from TDoH who administered both the polio immunizations and TB skin tests, and the clerical personnel from each organization who meticulously recorded, transcribed and processed the voluminous data and who carefully prepared our lengthy reports.

Recognition is due Herbert Pahren, USEPA (Cincinnati) for his foresight in recognizing the research potential of a health study at the Hancock site and his guidance in formulating the initial study design. Finally, we acknowledge the invaluable counsel and support provided by Walter Jakubowski and Dr. Dennis George. Their guidance and participation in the management of the LISS greatly exceeded the requirements of their respective responsibilities as project officer and contractor, and has been instrumental in its successful conduct.

SECTION 1

INTRODUCTION

A. BACKGROUND

Land Application and Potential Infectious Disease Hazards

Land application of wastewater can be an attractive alternative to traditional waste disposal practices. It avoids contamination of surface waters, provides additional waste treatment, returns nutrients to the soil, and reuses the water. The policy of the U.S. Environmental Protection Agency (EPA) is to ''press vigorously for publicly-owned treatment works to utilize land treatment processes to reclaim and recycle municipal wastewater'' (Costle, 1977). Applicants for federal construction grants (Section 201) must show in their requests that they have considered the application of wastewater to land as an alternative. Financial incentives are provided to encourage land application (Clean Water Act of 1977). Slow rate application of wastewater to land by spray irrigation has been and continues to be one of the most popular application methods. With EPA encouragement, it is likely that the practice of applying wastewater to land by sprinkler irrigation according to EPA design criteria (USEPA, 1977 and 1981) will become more prevalent as a means of final treatment and disposal.

Along with its considerable benefits, land application of wastewater entails the potential risk of infection from exposure to microorganisms in the wastewater. A variety of agents of human disease, including many overt and potentially pathogenic microorganisms, may survive treatment processes (Guentzel, 1978), and thus could theoretically pose a threat. There are various environmental pathways by which these agents in the wastewater and the aerosol produced by its sprinkler application might be introduced and initiate infection in susceptible exposed individuals. Farmers will come in direct contact with the wastewater and its sprayed mist in the course of their work with the irrigation system. Agents in the wastewater aerosol can be transported by the wind and might be inhaled or ingested in exposed food while still viable and infective. Other potential environmental pathways include: 1) ingestion of wastewater-contaminated ground water used as the domestic water supply, 2) dust storms in which wastewater-irrigated surface soils are entrained by strong winds, 3) insect vectors (e.g., flies attracted by the wastewater lagoons), 4) rodents (e.g., feed or food stuffs contaminated by fecal droppings or urine from field mice, infected by wastewater spray, which may be spending the winter in farmhouses and barns), and 5) fomites (e.g., wastewater-contaminated work shoes, clothing, hands, or doorknobs). Once introduced into the local population, the infectious agents might be transmitted by contact between infected and susceptible individuals.

Recent Literature

The study of Katzenelson et al. (1976) cautioned that the infectious disease hazards associated with irrigation of partially treated wastewater are greater than previously assumed. Existing illness records were analyzed in a retrospective study of enteric diseases among communal agricultural settlements (kibbutzim) in Israel. The incidence rates of enteric illness for kibbutzim utilizing wastewater for spray irrigation were compared with other kibbutzim practicing no form of wastewater irrigation. Two- to fourfold increases in the incidence of shigellosis, salmonellosis, infectious hepatitis, and typhoid fever were reported for the kibbutzim utilizing wastewater, whereas the incidence of other diseases not normally associated with sewage were similar in both groups. A subsequent retrospective study of Israeli kibbutzim by Shuval et al. (1983) identified serious deficiencies in the data of the original study, including misclassification of some kibbutzim regarding wastewater reuse, uncertainties about periods of irrigation, and the inadequacy of the communicable disease reports used as the basis for the study. Indeed, the subsequent study failed to find evidence of excess risk associated with wastewater irrigation except in kibbutzim in a ''switch'' category (i.e., in kibbutzim practicing two consecutive years of wastewater irrigation followed by the same period without irrigation or vice versa). In this category, a significantly increased risk of total enteric disease was noted only for the 0-4 age group during periods of wastewater irrigation.

Two prospective epidemiologic studies were conducted among residents around activated sludge sewage treatment plants near Chicago, Illinois using the family-based virus watch approach developed by Frost et al. (1941a,b) and Fox et al. (1957, 1966, 1972, 1974). Both studies included a health watch of participating households that involved health diaries, serology, and clinical specimen isolations. Neither Johnson et al. (1980) nor Northrop et al. (1980, 1981) detected any obvious adverse health effects in residents potentially exposed to wastewater aerosols from aeration basins.

Occupational health effects of wastewater and wastewater aerosols have also been investigated. A study by Linnemann et al. (1984) of Muskegon County, Michigan workers exposed to wastewater spray irrigation failed to show any differences in illness or viral isolation rates between the workers and a control group. Although antibody titers to coxsackievirus B5 were significantly higher in spray irrigation nozzle cleaners, seroconversions were not documented. Likewise, a prospective seroepidemiologic study by Clark et al. (1981) of municipal sewer and sewage treatment workers and controls in three American metropolitan areas failed to support a significant risk associated with exposure to the wastewater. However, inexperienced workers reported significantly higher rates of gastrointestinal illness, and the level of antibody to certain viruses appeared to be related to level of exposure to wastewater aerosols. In Sweden, Rylander and Lundholm (1980) found increased incidence of acute febrile illness among workers exposed to sludge dust (probably due to endotoxins) and also increased incidence of gastrointestinal symptoms among sewage treatment workers.

None of these studies has investigated the effects on nearby residents' health of sprinkler irrigation of wastewater over a known broad range of wastewater quality. The Lubbock Infection Surveillance Study (LISS) was designed to observe any association of the potential infectious disease effects with exposure to sprayed wastewater.

The Lubbock Land Treatment System (LLTS) Expansion

A major new land treatment system was constructed as a demonstration project (George, 1984) to apply wastewater from Lubbock, Texas by sprinkler irrigation at the Hancock farm near Wilson, Texas (see Figure 1). The design and operation of this large demonstration project provided for collection of research data under a wide range of quality of the wastewater that was used for irrigation. The first four major irrigation periods after the LLTS expansion commenced operation in February 1982 were monitored. The quality of the applied wastewater was substantially different in each of the four periods. The original spray nozzles directed the wastewater upward, which enhanced the creation and drift of aerosols. Thus, the LISS investigated the risk of wastewater exposure ranging from conditions representative of established guidelines [fecal coliforms <1000 MPN/100 mL (USEPA, 1981)] to those which explored the relative safety factor of the guidelines.

The LISS was one of several areas of research which were conducted simultaneously at the land treatment demonstration site. The chemical, biological and physical conditions of the ground water, soils, and crops were characterized prior to and during the wastewater irrigation (George et al., 1985a). The effects of hydraulic, nutrient, and salt mass loading were assessed on the percolate (Ramsey, 1985) and on the crops and soil (George et al., 1985b). George has provided a summary of all research findings (1985c).

The Lubbock Infection Surveillance Study (LISS)

The LISS was conducted to monitor infections in the community surrounding the new land treatment demonstration system. This prospective observational study has attempted to determine the association, if any, between the occurrence of infectious diseases in residents and workers and their exposure to the wastewater and aerosols produced by wastewater spray irrigation. The initial two years of operation of the LLTS expansion at the Hancock farm were investigated. LISS involved a 4-year health watch of nearby residents and microbiological monitoring of the wastewater and its aerosol. This site is unique in that a typical rural community with no prior wastewater exposure was challenged by the enteric agents active in a much larger urban community (Lubbock). Persons residing around the Hancock site may have been exposed to infectious agents indigenous in the Lubbock population but not circulating in the study area. Thus, many in the study population may have been relatively susceptible to the pathogens in the wastewater. A health watch of the rural community was maintained before, during, and after periods of wastewater spray irrigation. The health watch focused on infections detected serologically and through isolates recovered from routine fecal specimens. To enhance

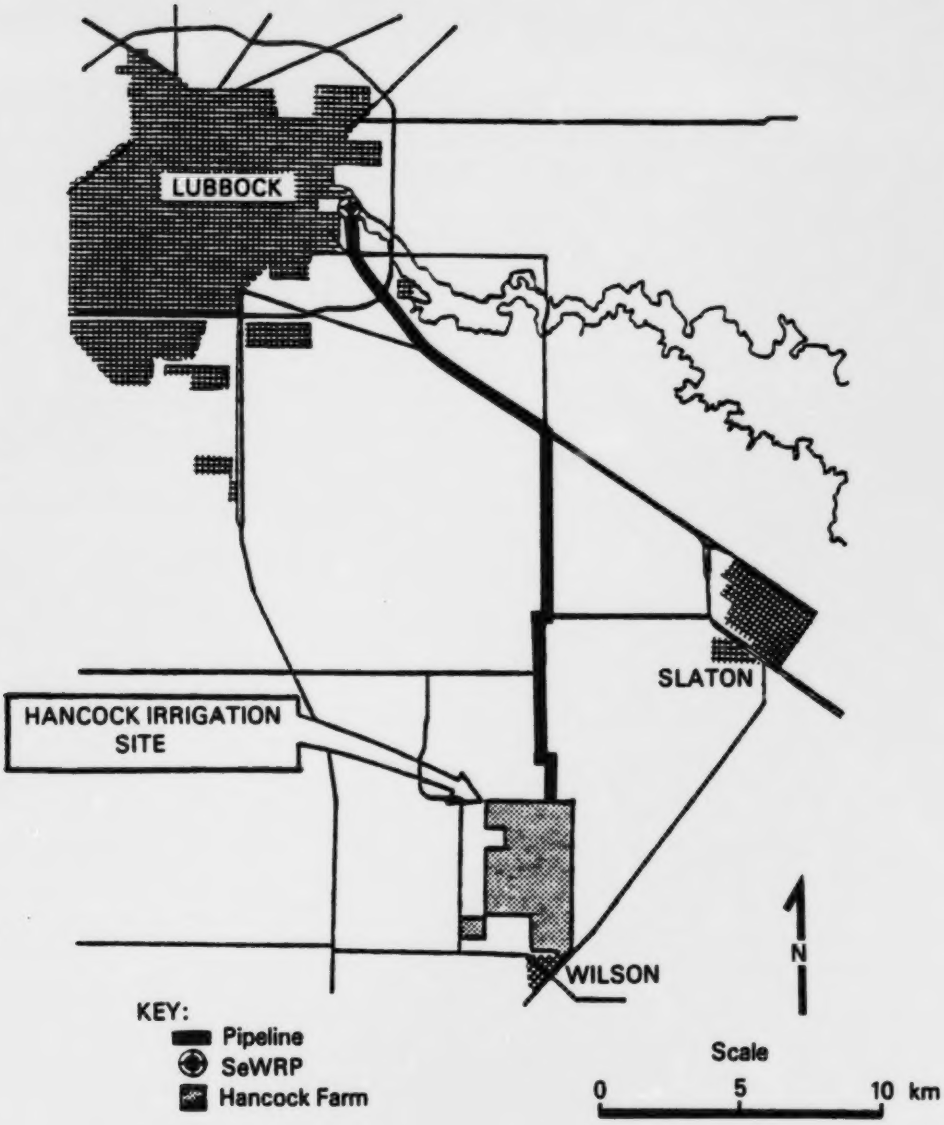

LUBBOCK
SLATON
HANCOCK IRRIGATION SITE
WILSON
N
KEY:
Pipeline
SeWRP
Hancock Farm
Scale
0
5
10 km

Figure 1. Wastewater irrigation system

the likelihood of interpreting observed episodes of infection, the likely routes of introduction and transmission were monitored.

B. STUDY OBJECTIVE

The general objective of the LISS was to identify possible adverse effects on human health from slow rate (sprinkler) land application of wastewater which contained potentially pathogenic microorganisms. More precisely, the objective was to determine the association, if any, between the occurrence of infectious diseases in residents and workers and their exposure to the wastewater and aerosols produced by wastewater spray irrigation. This objective was accomplished by disease surveillance of the study population, by description of the distribution of infections, and principally by evaluation of the incidence of infections for association with exposure.

C. STUDY DESIGN

The LISS was designed to monitor infections and illnesses occurring in the study population and concurrent environmental levels of the infectious agents as illustrated in Figure 2. The diseases, estimated susceptibility, and seasonal occurrence of the human pathogens potentially present in wastewater are summarized in Table 1. Disease surveillance was maintained to protect the population from any obvious untoward effects. However, the study focused on infections and the infecting agents rather than illness in order to obtain greater objectivity, sensitivity, specificity, and etiologic evidence.

All participants were asked to provide blood samples semiannually, usually in June and December. Sera were assayed for antibody titers to specific enteroviruses and other microorganisms known or suspected to be present in the sprayed wastewater. A seroconversion, defined as the fourfold or greater increase in agent-specific antibody titer in simultaneously tested successive sera from one individual, was considered serologic evidence that the individual had been infected by the agent during the time interval between the blood collections. Since mycobacteria were present in the wastewater, tuberculin skin tests were administered annually to give suggestive evidence of a non-tuberculosis mycobacterial infection.

An adult from each household and any children under 13 years of age were designated as fecal donors. Each donor, whether well or ill, was asked to submit routine stool specimens for microbiological testing during scheduled weeks which spanned each major irrigation period in 1982 and 1983. A series of three 1-week fecal collection sessions were scheduled before, during, and near the end of each irrigation period (see Figure 2) to detect infection events occurring in the interim. Clinical bacteriological analyses were performed to isolate overt and opportunistic pathogens. A semiquantitative measurement of growth (as heavy, moderate, light, or very light) was obtained by streaking primary plates by a four-quadrant method. Three categories of bacterial infection events were identified by comparing results from consecutive monthly specimens from an individual. Clinical virological analyses were performed to isolate enteric viruses in the fecal specimens by tissue culture techniques. Electron microscopic

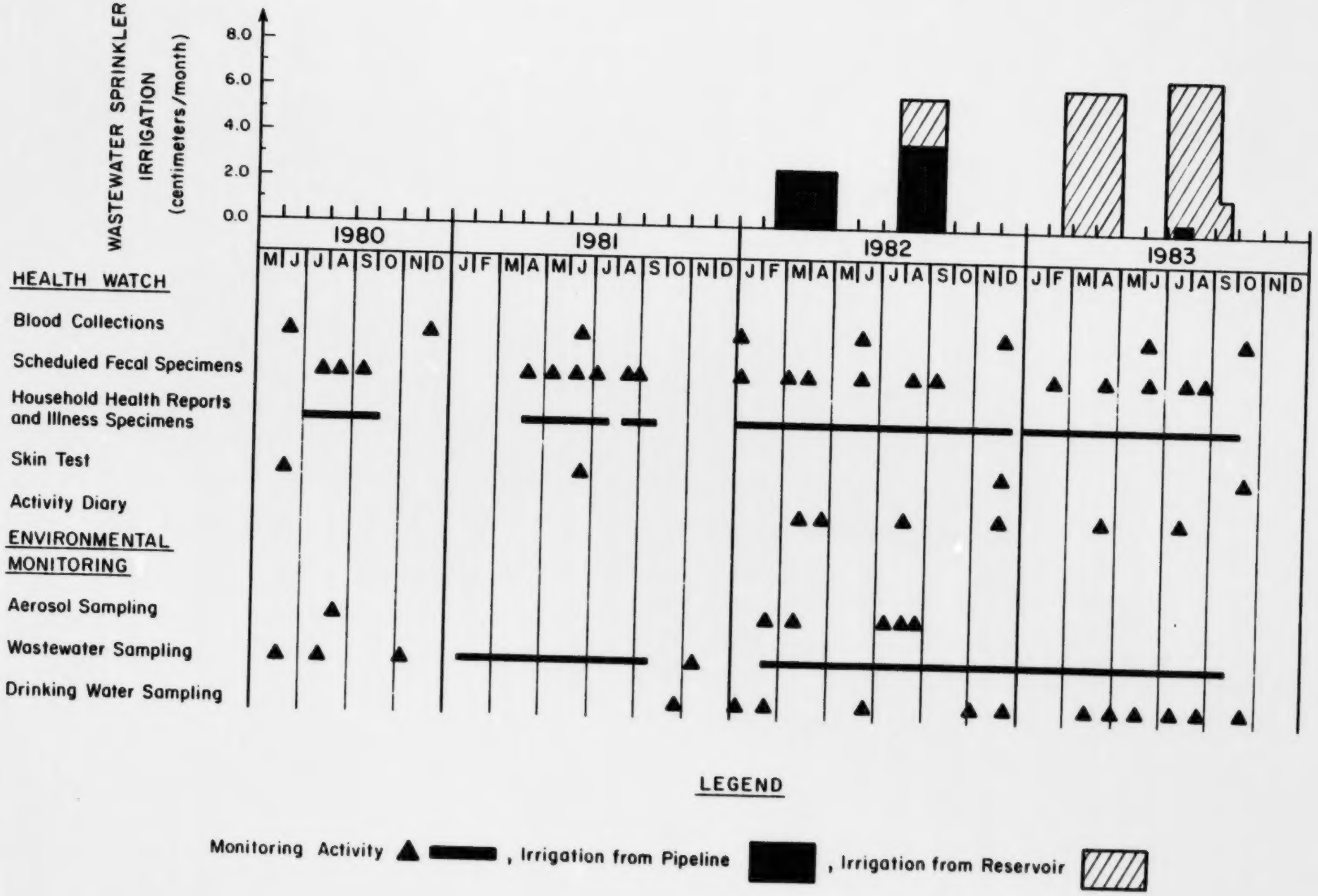


Figure 2. LISS study design: timeframe of monitoring in relation to major periods of irrigation

TABLE 1. SUGGESTED POPULATION SUSCEPTIBILITY AND SEASONAL OCCURRENCE OF INFECTION FOR AGENTS POTENTIALLY PRESENT IN WASTEWATER

Agent (human pathogens potentially present in wastewater)	Types	Disease	Percent of population susceptible	Time of occurrence											
				J	F	M	A	M	J	J	A	S	O	N	D
Viral															
Poliovirus[a]	1-3; wild and vaccine	Enteritis, meningitis, paralysis	<10% child	- -	- -	- -	- -	- -	—	—	—	—	- -	- -	- -
Coxsackievirus[a]	A 1-24, B1-6	Enteritis, meningitis, respiratory, rash	>50%						—	—	—	—			
Echovirus[a]	1-33	Meningitis, conjunctivitis	>50%						—	—	—	—			
Reovirus[b]	1-3	Unknown	>40%	—	—	—									
Adenovirus[a]	1-41	Respiratory	>50%	—	—	—								—	—
Hepatitis A virus[c]	1	Systemic	>70%					—	—	—	—	—	—		
Rotavirus[d]	1-4	Enteritis	>90%	—	—	—									—
Norwalk virus[d]	1-3	Enteritis	>50%							—	—	—	—		
Coronavirus[e]	2	Uncertain, enteritis	?	—	—	—	—	—							—
Bacterial															
Salmonella sp.[f]	10 groups	Enteritis, systemic	>75%						—	—	—	—	—		
Shigella sp.[g]	4 groups	Enteritis	>75%						—	—	—	—	—		
Escherichia coli, enteropathogenic[h]	Serotype O and other	Enteritis	>75%						—	—	—	—	—		
Mycobacteria, non-tuberculosis[i]	4 groups	Respiratory, adenitis, granuloma	>75%	—	—										—
Klebsiella pneumoniae[f]	5	5% respiratory, enteritis	>75%			—	—	—							
Yersinia enterocolitica[f]	4 biotypes	Enteritis, cutaneous	>75%						—	—	—	—	—		
Campylobacter sp.[j]	4 or more	Enteritis, systemic	?												
Legionella pneumophila[k]	23 or more	Respiratory, renal, other	>90%								—	—	—		
Staphylococcus aureus[f]		Respiratory, enteric, cutaneous	>75%	—	—	—	—	—	—	—	—	—	—	—	—
Streptococcus beta, hemolytic[f]	4 of 15 candidates	Respiratory, enteric	>75%	—	—	—	—	—	—	—	—	—	—	—	—
Pseudomonas sp.[f]	7	Cutaneous, respiratory, other	<25%	—	—	—	—	—	—	—	—	—	—	—	—
Proteus sp.[f]	3 or more	Cutaneous, respiratory, other	<25%	—	—	—	—	—	—	—	—	—	—	—	—
Fungal															
Candida albicans[f]	A, B groups	Cutaneous, respiratory, other	<25%	—	—	—	—	—	—	—	—	—	—	—	—

References:

a Fox and Hall (1980); b Jackson and Muldoon (1973b); c Szmuness et al (1977); d Cukor and Blacklow (1984); e Gerna et al (1985); f Lennette et al (1985); g Black et al (1978); h Sack (1975); i Ahn et al (1979); j Blaser et al (1983); k Brenner (1984) examination was performed on about 1/4 of the routine fecal specimens to detect a variety of virus-like particles, many of which are not recoverable by tissue culture techniques. Detection of a specific virus by laboratory cultivation or by electron microscopic examination was considered evidence of a viral infection. Each non-adenovirus viral infection was regarded to be new, unless the same agent had been recovered from the individual in the prior 6 weeks.

Each household was contacted weekly by telephone for a report of any illnesses during the prior week. When a sufficiently recent respiratory or gastrointestinal illness was reported, the ill participant was requested to submit a throat swab or stool specimen to identify the causative agent. Weekly self-reports of illness and appropriate illness specimens were obtained over the entire period of irrigation from January 1982 until October 1983 and over baseline periods corresponding to seasons of heavy irrigation.

The types and densities of potentially pathogenic bacteria and viruses were monitored in the wastewater, wastewater aerosol, and other environmental routes of introduction and transmission. An effort was made to determine the fluctuations in levels of every measurable infectious agent utilized in the health watch, as indicated in Table 2. However, the low densities of many agents in environmental samples necessitated reliance on indicator organisms to establish environmental patterns. Wastewater samples of the effluent from the pipeline and reservoirs to be utilized for spray irrigation, and of the Wilson effluent, were obtained and analyzed for indicator bacteria and enteroviruses biweekly to span the major irrigation periods; corresponding baseline samples had been obtained with the same frequency in 1981 and at lesser frequency in 1980 to characterize the effluents. Microbiological screens of indigenous enteric bacteria were conducted on one sample each from the pipeline and the reservoir per irrigation season. The purpose of the routine wastewater samples was to document the presence, prevalence, longitudinal pattern, and passage through the study community of viral and bacterial pathogens possibly introduced by the wastewater. Extensive aerosol sampling was conducted to characterize the aerosol density of indicator microorganisms produced by the spray irrigation of both pipeline and reservoir wastewater. Virus runs were also conducted to measure the density and diversity of enteroviruses in aerosols emanating from the sprinkler rigs. Drinking water, houseflies, and dust storms also were evaluated as other means of introducing microorganisms into the study population.

An aerosol exposure index (AEI) was devised to measure the degree of a participant's cumulative exposure to microorganisms in the wastewater aerosol, relative to all other study participants during a given irrigation period. When a number of similar infection events were observed either serologically or microbiologically in the study population within a time interval corresponding to an irrigation period, this infection episode was statistically analyzed for association with wastewater aerosol exposure using AEI. Infection incidence rates were compared among exposure subgroups and with baseline rates to determine the relative risk of infection.

TABLE 2. FREQUENCY OF MEASUREMENT IN WASTEWATER OF INTERPRETABLE INFECTIOUS AGENTS MONITORED IN THE HEALTH WATCH

Agents monitored in health watch		Measurement in wastewater		
Procedure	Infectious agents (serotypes potentially present in wastewater)	Sprayed wastewater	Wilson wastewater	Data type
Serology	(total enteroviruses: coxsackie, echo, polio)	R	R	Q
viruses:	Coxsackie A virus (1-24)	R	R	S (by ID)
	Coxsackie B virus (1-6)	R	R	S (by ID)
	Echovirus (1-33)	R	R	S (by ID)
	Adenovirus (1-9, 11, 19, 21)			
	Reovirus (1-3)			
	Hepatitis A virus			
	Rotavirus (1-4)			
	Norwalk virus (1-2)			
bacteria:	Legionella pneumophila	I		+/- (will ID)
Skin Test	Mycobacteria (tuberculosis + non-tuberculosis)	R	R	Q
Clinical Bacteriology				
bacteria:	Salmonella sp.	R	R	+/-
	Shigella sp.	R	R	+/-
	Yersinia enterocolitica	R	R	+/- (Q if high)
	Campylobacter jejuni	R	R	+/-
	Staphylococcus aureus	I	I	Q
	Fluorescent Pseudomonas	R	R	Q
	Klebsiella	R (K1-like)	R	Q
	Proteus	I	I	Q
	Serratia and others	I	I	Q
	Aeromonas hydrophila	I	I	S
fungus:	Candida albicans	R	R	Q
Clinical Virology	Polioviruses	R	R	S (by ID)
	Coxsackie A virus (1-24)	R	R	S (by ID)
	Coxsackie B virus (1-6)	R	R	S (by ID)
	Echoviruses (1-33)	R	R	S (by ID)
	Adenoviruses (by group antigen)			

R - regular
I - infrequent

Q - quantitative
S - semiquantitative

+/- - present/absent
ID - identification

D. STUDY ORGANIZATION

The LISS involved five major functional activities: project management, a health watch, environmental sampling, microbiological assay of clinical specimens and environmental samples, and data analysis. The field activities (i.e., health watch, environmental sampling, and their management) were funded by a subcontract to SwRI from LCCIWR (SwRI Project 01-6001). The other activities (i.e., laboratory analysis, data analysis, and their management) were funded by a cooperative agreement between EPA-HERL and SwRI (SwRI Project 01-6097).

The LISS was conducted by Southwest Research Institute, the University of Illinois at Chicago, and the University of Texas at San Antonio and Austin. The following is a listing of participating organizations:

Southwest Research Institute (SwRI)
Department of Environmental Sciences
San Antonio, Texas

University of Illinois at Chicago (UI)
School of Public Health
Chicago, Illinois

University of Texas at San Antonio (UTSA)
Center for Applied Research and Technology (CART)
San Antonio, Texas

University of Texas at Austin (UTA)
Austin, Texas

U.S. Environmental Protection Agency
Health Effects Research Laboratory (EPA-HERL)
Cincinnati, Ohio

Lubbock Christian College
Institute of Water Research (LCCIWR)
Lubbock, Texas

University of Texas
School of Public Health (UTSPH)
Houston, Texas

Naval Biosciences Laboratory (NBL)
Oakland, California

H. E. Cramer Company (HEC)
Salt Lake City, Utah

Texas Department of Health (TDoH)
Public Health Region 2
Lubbock, Texas

Illinois Department of Public Health (IDPH)
Laboratory Section
Chicago, Illinois

Centers for Disease Control (CDC)
Atlanta, Georgia

University of Massachusetts (UM)
Worcester, Massachusetts

Metpath Laboratories
Des Plaines, Illinois

The project manager for the LISS was Mr. David E. Camann, SwRI. Each of the functional activities was directed by a principal investigator who reported to Mr. Camann as shown in Figure 3. Details regarding principal participating personnel, participating organizations, and areas of specific activity are presented in Table 3 for each functional activity area.

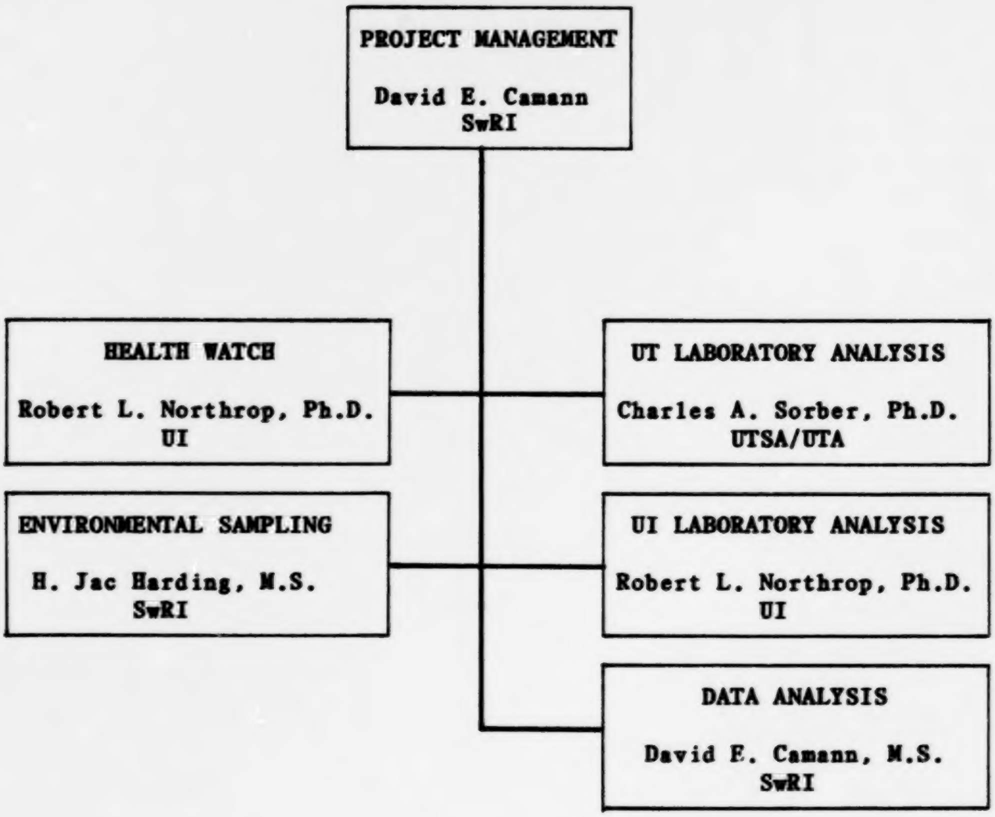

PROJECT MANAGEMENT
David E. Camann
SwRI
HEALTH WATCH
Robert L. Northrop, Ph.D.
UI
UT LABORATORY ANALYSIS
Charles A. Sorber, Ph.D.
UTSA/UTA
ENVIRONMENTAL SAMPLING
H. Jac Harding, M.S.
SwRI
UI LABORATORY ANALYSIS
Robert L. Northrop, Ph.D.
UI
DATA ANALYSIS
David E. Camann, M.S.
SwRI

Figure 3. Principal investigators and functional areas

TABLE 3. PRINCIPAL PARTICIPATING PERSONNEL AND AREAS OF ACTIVITY

Personnel	Organization	Specific activity areas
PROJECT MANAGEMENT (D.E. Camann, SwRI)		
D.E. Camann	SwRI	Planning, technical and financial status, meetings, reports
R.J. Prevost	SwRI	Administration of subcontracts
H.J. Harding	SwRI	Annual reports
J.K. Moravits	SwRI	Report preparation
A. Shelokov	Johns Hopkins	Consultant (study design)
A. Holguin	UTSPH	Consultant (epidemiology)
HEALTH WATCH (R.L. Northrop, UI)		
P.J. Graham/C.M. Becker	UI	Recruitment, health surveillance, serum and specimen collection, household health and activity diary collection
I. Smith/S. Stebano/J. Steinhauser		On-site coordinator, Wilson, Texas
C.R. Allen	TDoH	Polio vaccination, tuberculin testing
ENVIRONMENTAL SAMPLING (H.J. Harding, SwRI)		
H.J. Harding	SwRI	Wastewater aerosol sample collection, wastewater and meteorological sampling
M.A. Chatigny	NBL	Loan and calibration of LVA samplers
S. Schaub	US Army Ft. Detrick	Loan of Andersen samplers
D.B. Leftwich/N. Klein	LCCIWR	Sample collection
LABORATORY ANALYSIS (C.A. Sorber, UTSA/UTA, R.L. Northrop, UI)		
Environmental Samples		
B.E. Moore/C.A. Turk/ M. Iberra	UTSA/UTA	Analysis of wastewater samples (microbiological screens, routine wastewater assays; enterovirus identification) Analysis of aerosol and fly samples
D.B. Leftwich	LCCIWR	Analysis of drinking water
R.L. Northrop/ R. Cordell	UI	Analysis of Legionella in wastewater
B.P. Sagik	Drexel Univ.	Consultant (virology)
Clinical Specimens		
		Serology
P.J. Graham	UI	Poliovirus, coxsackievirus, echovirus, adenovirus
R. Cordell	UI	Reovirus, rotavirus, influenza A
W. Nunes	UI	Legionella bacillus
B.E. Moore/R. DeCresce	UTSA/Metpath	Hepatitis A
N.R. Blacklow	UM	Norwalk virus
G.R. Healy	CDC	E. histolytica
B.E. Moore/C.A. Turk	UTSA/UTA	Clinical virology
M.N. Guentzel/ C. Herrera	UTSA	Clinical bacteriology
W. Jakubowski/ F. Williams	EPA-HERL	Electron microscopy of fecal specimens
C. Sweet	TDoH	Ova and parasite analysis
R. Murphy	IDPH	Consultant (serologic methods)
M.K. Cooney	Univ. Wash.	Consultant (serology)
DATA ANALYSIS (D.E. Camann, SwRI)		
K.T. Kimball	SwRI	Statistical analysis
R.L. Mason/ J. Buckingham	SwRI	Logistic regression analysis
J. Garza/M. Camann	SwRI	Data base management
N. Altman	UI	Data management
D.E. Camann	SwRI	Aerosol exposure, bacterial and viral infection patterns
P.J. Graham	UI	Seroconversion incidence, illness patterns
A. Anderson	HEC	Dispersion modeling
R. Harrist	UTSPH	Consultant (statistical methods)
J. Stober	EPA-HERL	Consultant (statistical methods)

SECTION 2

CONCLUSIONS

1. The LISS employed an epidemiologic analytic prospective cohort study design which was quite appropriate to measure the strength of association between exposure to the wastewater used for irrigation and the development of new infections. The results from the isolation and serology procedures used to detect infections appear to be adequate. These detection methods were sufficiently sensitive and specific to observe many episodes of infection in the study population in which the etiologic agent was identified. The size of the population was sufficient to analyze the distribution of observed infections for possible association with exposure to wastewater irrigation and to control for extraneous variables via logistic regression analysis. However, the small population size led to instability of the association. The significance of the study findings have not been limited to a great extent by such major confounding factors as age, gender, antibody level, head of household education, and time spent in Lubbock.

2. The quality of the wastewater to which the study population was exposed was highly variable during the study. During the initial spring 1982 irrigation period, the quality of the irrigation wastewater approximated that of a low quality primary effluent, as determined by physical and chemical analyses. While the quality of the irrigation wastewater was greatly improved in 1983, its fecal coliform concentration still exceeded the EPA guideline for controlled agricultural irrigation as practiced at the study site.

3. Spray irrigation of wastewater obtained via pipeline directly from the Lubbock SeWRP was a more substantial source of aerosolized microorganisms than spray irrigation of wastewater stored in reservoirs. Enteroviruses were consistently recovered in the aerosol at 44 to 60 m downwind of irrigation with pipeline wastewater.

4. Microorganism levels in air downwind of spray rigs using pipeline wastewater were significantly higher than upwind levels: fecal streptococci levels to at least 300 m downwind, and levels of fecal coliforms, mycobacteria and coliphage to at least 200 m downwind. Levels downwind were also significantly higher than background levels in ambient air outside of participants' homes: fecal coliform levels to beyond 400 m downwind, mycobacteria and coliphage levels to at least 300 m and fecal streptococci levels to at least 200 m.

5. The exposure which most of the study population received to most microorganisms via the wastewater aerosol was greater in 1982 than in 1983. The cumulative enterovirus dose received from aerosol exposure at

a given distance downwind in summer 1982 was estimated to be at least an order of magnitude greater than in any other irrigation period.

6. Individuals in the high (AEI≥3) and low (AEI<3) exposure groups were generally well balanced with regard to infection risk factors, including age, gender and previous antibody titer. The high exposure fecal donors ate food prepared by a local restaurant very significantly more often, made greater use of evaporative coolers for air conditioning, and had more farmers as head of household.

7. The lack of a strong, stable association of clinical illness episodes with the level of exposure to irrigation wastewater indicates that wastewater spray irrigation did not produce obvious disease during the study period. However, the participants in the high exposure level (AEI>5) reported a slight excess crude incidence density of total acute illness shortly after the onset of wastewater irrigation, both in spring 1982 and in summer 1982, the seasons of initial and heaviest microbial exposure, respectively. The extent to which this reflects actual illness versus possible reporting bias by high exposure participants cannot be ascertained.

8. The occurrence of enteric Gram-negative bacteria (EGNB) at moderate and heavy levels in the throats of both healthy and ill study participants was frequent and widespread between July 19 and October 12, 1982. The household environment was strongly associated with the continuing EGNB throat infections of one household. Among the ill throat swab donors, use of an evaporative cooler for home air conditioning was associated with the EGNB throat infections.

9. Some excess risk of viral infection (risk ratio of 1.5 to 1.8) was associated with wastewater aerosol exposure, based on comparison of crude seroconversion incidence densities by aerosol exposure level and by irrigation vs. baseline period.

10. A symmetric risk ratio score approach provided evidence of a stable and dose-related association between infection events and wastewater aerosol exposure in the infection episodes observed by the LISS.

11. Some infection episodes appear to have been related to wastewater aerosol exposure, because more statistically significant associations than expected were found in the confirmatory analysis of independent infection episodes using a one-sided Fisher's exact test. Some imbalances in the two populations may provide alternate explanations for the excess associations. On the other hand, the number of detected increases in incidence rates associated with the wastewater irrigation may be underestimated, considering the relatively modest power of the tests to detect small differences.

12. An exploratory logistic regression analysis found significant ($p<0.05$) associations between presence of infection and degree of aerosol exposure while controlling for the effects of extraneous variables in four infection episodes. More supporting evidence was found for the wastewater

aerosol route of exposure than for direct contact with wastewater or spending time in the irrigation environment on the Hancock farm.

13. Eight specific infection episodes displayed good or marginally consistent evidence of association with wastewater aerosol exposure.

    a. Two of these episodes were probably unrelated to wastewater exposure because a more plausible alternative explanation was identified:

        - o Episode of Klebsiella infections in summer 1983
          —alternative: eating at a local restaurant

        - o Spurious control episode of echovirus 9 seroconversions in the baseline period
          —alternative: within household spread

    b. The evidence is inconclusive in five episodes because both aerosol exposure and the identified alternative explanation(s) are plausible risk factors:

        - o Episode of clinical viral isolates excluding adenoviruses and immunization-associated polioviruses in summer 1982
          —alternative: eating at a local restaurant

        - o Episode of echovirus 11 seroconversions in 1982
          —alternatives: o contaminated drinking water
          o caucasian, large household

        - o Episode of seroconversions to viruses isolated from wastewater in summer 1982
          —alternatives: o contaminated drinking water
          o low income, caucasian

        - o Episode of seroconversions to viruses isolated from wastewater in 1982
          —alternative: farmer, history of pneumonia

        - o Episode of seroconversions in summer 1982 to all serum neutralization-tested viruses
          —alternative: contaminated drinking water

       All five of these infection episodes relate to echo or coxsackie B viral infections observed primarily in summer 1982 and primarily to agents recovered from the wastewater at that time.

    c. Some of the infections in one episode were probably caused by wastewater aerosol exposure because a strong association existed and no alternative explanation could be identified:

        - o Episode of poliovirus 1 seroconversions in spring 1982

Three distinct risk factors (poliovirus immunization in spring 1982, low polio 1 antibody titer in January 1982, and a high degree of aerosol exposure) were independently associated with the poliovirus 1 seroconversions in spring 1982 and each appears to have been responsible for some of the poliovirus 1 infections.

14. Despite the efforts to obtain a random sample, the study participants during the irrigation periods were essentially volunteers who were not representative of the entire population of the study area. Furthermore, the frequency of patronizing local restaurants and the use of evaporative coolers were factors that were largely confounded with wastewater aerosol exposure. For these reasons, the LISS findings cannot easily be generalized to other sites.

15. In summary, a general association existed between exposure to irrigation wastewater and new infections. A viral dose-response relationship was observed over the four irrigation seasons, since the aerosol exposure-associated episodes of viral infection occurred primarily in 1982 during the irrigation seasons of greater enterovirus aerosol exposure. Some poliovirus 1 seroconversions during the spring of 1982 were probably related to wastewater aerosol exposure. However, even during 1982, the strength of association remained weak and frequently was not stable. Wastewater of poor quality comprised much of the irrigation water in 1982. Of the many infection episodes observed in the study population, few appear to have been associated with wastewater aerosol exposure, and none resulted in serious illness.

SECTION 3

RECOMMENDATIONS

1. To minimize exposure, it would be prudent to use wastewater from the reservoirs at the Hancock farm for irrigation (or to apply equivalent treatment measures), rather than irrigating directly from the pipeline.

2. Poliovirus serology should be performed on archived sera from June 1982 through October 1983 to identify poliovirus seroconversions in the study population spanning the summer 1982 and the 1983 irrigation periods. Any observed poliovirus infection episodes should be fully analyzed by the inferential methods employed in the LISS. Since summer 1982 and possibly summer 1983 appear to have been seasons of higher poliovirus aerosol exposure than spring 1982 was, these data would confirm or dispute the probable relationship of poliovirus 1 seroconversions to wastewater aerosol exposure which was observed in spring 1982.

3. Serological testing of archived sera is recommended for selected enteroviruses and rotavirus to observe and analyze additional infection episodes in order to clarify the apparent dose-response relationship with wastewater aerosol exposure detected in the LISS.

    a. Perform serum neutralization retesting to improve existing infection episode data. There are 56 echovirus and adenovirus infections reported for the years 1982 or 1983 that need additional serologic testing to identify the exact 6-month interval in which the seroconversion occurred. Also, there were 28 serologic series in which infection status was indeterminate due to inconsistent or contradictory titer results and 33 unconfirmed four-fold or greater titer rises in unpaired sera; these cases were not used in the LISS data analysis.

    b. Conduct rotavirus and coxsackie B virus serology having a high probability of yielding additional infection episodes to agents found in sprayed wastewater. Rotavirus serology should be performed on the entire serum donor population, since a very high incidence density of seroconversions to rotavirus was observed throughout the study period in both the 45 children and the 11 adults tested in the LISS. Additional serology testing for coxsackieviruses B2, B3 and B4 is recommended based on their recovery from the wastewater in 1982 and 1983.

    c. Serologic testing of echoviruses 12, 25, 27 and 31 is recommended, because they were each recovered from wastewater in several of the irrigation periods.

4. An exposure assessment should be performed to estimate the range of cumulative organism exposure dosages that applied to the LISS infection episodes and other situations in which reasonable evidence of association with wastewater irrigation was obtained. A dosage to the infectious agent should be estimated for each infected individual and the dosage range of the high exposure level of participants should be approximated. Determination of the dosage range in which observed infection effects were found would provide a crucial missing link in the relationship between viable aerosol concentration and infection. This would facilitate transfer the dose-response findings of the LISS to other sites of wastewater aerosol exposure.

5. An improved model of microbiological dispersion should be developed based on the LISS aerosol sampling data. The LISS data provide a much better basis for model development than the data bases previously employed. The model would permit the determination of the estimated range of microorganism exposure dosages at considerable distances downwind (i.e., 400-800 m) from any spray irrigation source of wastewater aerosols.

6. If recommendation 1 is not implemented, a limited program of wastewater and aerosol sampling should be conducted at the Hancock farm to determine densities of enteroviruses and indicator bacteria in wastewater and downwind air and to reevaluate aerosolization efficiency for the current treatment process and mode of operation. ''Pulsed break-point chlorination'' of pipeline wastewater and installation of proper spray nozzles to reduce aerosol formation and drift are two major changes in irrigation practices at the Hancock farm since 1983. The sampling program would permit determination of where the current irrigation practices fit into the seasonal dose-effect gradient found in the LISS.

7. It is recommended that analyses of existing LISS data be performed as pilot studies to investigate whether clinically and serologically detected infections and self-reported illness were associated with several apparent environmental sources of infection identified in the LISS:

    a. Evaluate bacterial contamination of wells which served as sources of household drinking water.

    b. Evaluate patronage of local restaurants in this rural community to help to address the extent to which food prepared for public consumption may be a source of inapparent infections and minor acute illness.

    c. Evaluate the use of evaporative coolers for air conditioning as a source of bacterial infections and illness, especially when bacterial contamination of water supplies is quite widespread.

8. Certain additional data analyses are recommended to facilitate proper interpretation of the LISS results:

   a. Calculate incidence density ratios and their confidence intervals for clinical agents, as was done for serologic agents and self-reported illness, in order to balance the procedure for selection of infection episodes with good and marginal evidence of association with aerosol exposure.

   b. Investigate the need to control by logistic regression analysis for the effects on infection status of three additional factors which were partially confounded with wastewater aerosol exposure: evaporative cooler use prior to 1983, rural versus Wilson location, and children in the household.

   c. Conduct a stratified analysis of serologic and illness incidence densities to control for major potential risk factors, such as age, gender, previous antibody titer, occupation and education of head of household, restaurant patronage, and dwelling location. These analyses would clarify interpretation of apparent associations with aerosol exposure of seroconversions and self-reported illness which were based on test-based confidence intervals of crude incidence density ratios.

   d. Determine if there is evidence of association of infections with residential aerosol exposure when the individuals with occupational exposure to wastewater irrigation are excluded from the study population.

SECTION 4

METHODS AND MATERIALS

A. STUDY SITE

Description of Study Area

The Lubbock Land Treatment System is located in Lynn and Lubbock Counties in northwestern Texas. The source of wastewater for this irrigation project was the Lubbock Southeast Water Reclamation Plant (SeWRP), situated in the southeast portion of the city of Lubbock. The storage and irrigation facilities were located at the Hancock farm in the north central portion of Lynn County, 29 km (18 miles) south of Lubbock. Both counties are located in a plateau area, the South Plains Region of the Llano Estacado of the High Plains. A regional map of the study area was shown in Figure 1.

Lubbock is the center of the largest cotton producing section of Texas. Other segments of the agroeconomy of the area included grain sorghum production and cattle feeding. The Ogallala aquifer, an extensive unconfined aquifer system stretching from western Nebraska and eastern Colorado south to the Texas panhandle and eastern New Mexico, has been used for irrigation purposes as a supplement to natural rainfall to improve crop yields. Withdrawal of ground water from the Ogallala aquifer has greatly exceeded the natural recharge. In the Lubbock area, the aquifer is approaching depletion; in 15 years it may no longer be economical to produce irrigation water from this source.

General Climatology--

The South Plains Region is semiarid, transitional between the desert conditions to the west and the humid climate to the east and southeast. The average annual precipitation is 46.8 cm (18.4 inches), most of which occurs from May through September, usually as moderate to heavy afternoon and evening thunderstorms which may be accompanied by hail. Snow may occur from late October until April, but is generally light and seldom remains on the ground for more than 2 or 3 days at any one period.

During the 8-month period from March through October, winds are predominantly from the south. However, during the late winter and springtime, winds in excess of 11 meters/second (25 MPH) occur for periods of 12 hours or longer from a westerly direction with the passage of low pressure centers. These strong winds bring widespread dust, the quantity and amount of which is influenced by the precipitation patterns of the previous few days and the agricultural practices of the area (NOAA, 1977).

To anticipate the distribution of wind directions during the major irrigation periods, wind roses, based on Lubbock Airport data for 1969-1973, were constructed for those months. The wind roses for March and April (spring irrigation) and for July and August (summer irrigation) are shown in Figures A.1 and A.2, respectively, in Appendix A.

City of Wilson--

The City of Wilson was the nearest community to the Hancock farm. It was situated at the southern boundary of the farm. The population of 576 (1980 census) occupied 181 residences ranging from small two bedroom stucco or frame bungalows to large all-brick homes. Local commerce was based primarily on agriculture. Support facilities located in Wilson included three cotton gins, one grain elevator, a welding and machine shop, a pump service facility, and a combined lumber, hardware and feed store. Other businesses within Wilson included a bank, two cafes, two service stations, and a grocery store. During 1982 the grocery store ceased to do business and one service station was converted into a convenience store. A municipal building, a school complex for grades 1 through 12, a municipal park, a post office and six churches were also located within the city limits. There were no day care centers or medical facilities in the city.

The municipal water supply for city residents was obtained from six wells which tapped the Ogallala aquifer. A water tower and underground tank provided storage facilities where the water was intermittently chlorinated manually prior to distribution. Continuous chlorination of the City of Wilson water supply system commenced in March 1983.

All but ten of the households within the city limits were serviced by a municipal wastewater collection and treatment system. The treatment plant consisted of an Imhoff tank preceded by a bar screen. Plant effluent was allowed to evaporate from a series of lagoons while the settleable solids were removed from the tank on a monthly basis and placed in an adjacent drying bed. Those households not connected to the municipal system had septic tanks.

Rural Area--

The rural portion of the study area (see Figure 6) lay primarily in Lynn County (1980 census population, 8,605), with a small portion above the northern boundary in Lubbock County. Approximately 130 households were located in this area in 1980 with an estimated population of 450.

Almost every rural household obtained its drinking water from a nearby private well which tapped the Ogallala aquifer. Treatment of domestic wastewater was accomplished by septic tank systems in half of the rural houses while the other half, typically the older homes, utilized cesspools.

In the predominantly agricultural economy of this region, an annual income of $37.3 million (Lynn County) was derived from a primary crop of cotton and secondary crops of winter wheat, grain sorghum, sunflowers and soybeans. Livestock was kept primarily for owner use, though some pasture land was dedicated to grazing of livestock for market. There was some production of oil and gas, and some exploration, with attendant drilling activity, occurring in the area. The value of these mineral resources and those of a stone quarry amounted to $2 million during 1977 for Lynn County (Texas Almanac, 1980).

Lubbock Sewage Treatment Plants

The City of Lubbock operated two wastewater treatment plants: the Southeast Water Reclamation Plant (SeWRP) and the Northwest Water Reclamation Plant. The SeWRP was in reality three separate systems: two trickling filter plants (Plants 1 and 2) and an activated sludge plant (Plant 3). Due to the predominantly agricultural economic base of the Lubbock area, domestic sewage comprised the bulk (i.e., about 70%) of the wastewater treated by the SeWRP. The majority of industrial wastes were from cotton gin operations and industrial plating operations. Industries on a surcharge contract with the city contributed approximately 22% of the total 5-day biochemical oxygen demand (BOD_5) mass loading and 15% of the total suspended solids (TSS) mass loading to the SeWRP. An electroplating plant's discharge contained high levels of chromium (42 ppm average) and nickel (17.2 ppm average) and contributed the highest mass loading of heavy metals during the project period.

Trickling Filter Plant 1 had a hydraulic capacity of approximately 23,000 m^3/day (6 mgd). Plant 1 provided most of the water for the Gray farm, a 1,489 ha farm located east of the City of Lubbock, which comprised the older part of the Lubbock Land Treatment System.

Trickling Filter Plant 2 was designed to treat a maximum flow of 76,000 m^3/day. Normal flow ranged from 30,000 to 49,000 m^3/day (8 to 13 mgd). During 1980 and 1981, the effluent from Trickling Filter Plant 2 had a composition equivalent to a typical medium untreated domestic wastewater as defined by Metcalf and Eddy (1979). This poor quality effluent was mainly attributable to the malfunctioning of the anaerobic digestion process since effective liquid-solid phase separation was not achieved in the second stage digester. Consequently, the suspension recycled from the anaerobic process to the head works of the trickling filter plant contained high levels of ammonia, suspended solids and carbonaceous material. From June 1980 to February 1982, the average effluent total organic carbon (TOC) produced from Trickling Filter Plant 2 was 117.7 mg/L. Total Kjeldahl nitrogen (TKN) concentration averaged 38.59 mg-N/L of which 67% was ammonia-nitrogen (25.95 mg-N/L) and 33% was organic nitrogen. Due to high organic mass loadings and subsequent heterotrophic organism activity, the trickling filter system was not nitrifying ammonia to nitrate. Approximately 57% of the total phosphorus (14.43 mg/L) present in the effluent from Plant 2 was orthophosphate phosphorus (PO_4). Plant 2 provided the majority of the water pumped to the Hancock farm.

Treatment Plant 3, an activated sludge system, had a maximum design hydraulic capacity of 55,000 m^3/day (15 mgd). Effluent quality was fairly good with a BOD_5 of 25 mg/L and TSS of 18 mg/L. The effluent was dosed with about 12 mg/L chlorine. Southwestern Public Service (SPS, a power utility) utilized a major portion of this effluent as cooling and boiler makeup water. The effluent discharge not utilized by SPS (daily average of less than 5%) was divided equally between the Gray and Hancock land application sites.

The Northwest Wastewater Reclamation Plant treated wastewater generated mainly from the extreme northwest portion of Lubbock and from Texas Tech University. The 4,000 m^3/day (1 mgd) effluent from this plant was used by Texas Tech University for irrigation studies on the university farm.

Lubbock Land Treatment System

The original component of the Lubbock Land Treatment System (LLTS) was the Gray farm which has utilized effluent to grow crops since 1938. As the wastewater discharge increased due to population growth, the Gray farm was expanded to treat the increased hydraulic and nutrient mass loading. Eventually, insufficient land was available to adequately assimilate the hydraulic flow which resulted in a significant rise in ground water level and subsequent degradation of water quality. Therefore, the Hancock farm was included in the LLTS to reduce the hydraulic and nutrient overloading experienced at the Gray farm. In November 1980, construction began on a pump storage and distribution system to divert 50% of the total flow pumped to the Hancock farm, a new component of the LLTS.

The total cultivated area of the LLTS land application system was 2,565 ha during the period of study. The Gray farm, located east of the City of Lubbock, had a total land area of 1,489 ha with about 1,210 ha in cultivation. The 1,478-ha Hancock farm was located 27 km (17 miles) south of the SeWRP and just north of the community of Wilson. During the 5-year period from 1977 to 1982, the Hancock farm was primarily a dry land farm with little ground water irrigation.

The completely new system constructed at the Hancock farm consisted of wastewater conveyance, storage and irrigation facilities. The conveyance system consisted of a three-pump pumping station located adjacent to the existing effluent pumping station at the Lubbock SeWRP and 25 km of 0.69 m force main to the Hancock farm. The pumping station and the force main were designed to accommodate a flow of 28,000 m^3/day (7.4 mgd). The average wastewater flow was 14,000 m^3/day in 1982 and 1983.

At the northern boundary of the Hancock farm, the effluent was routed through three 0.38-m plastic irrigation pipelines to a storage system consisting of three separate reservoirs. These were constructed on natural playa lakes with capacities of 1.5×10^6 m^3 (Reservoir 1-east), 6.9×10^5 m^3 (Reservoir 2-central), and 7.4×10^5 m^3 (Reservoir 3-west). Irrigation pump stations were provided at each reservoir. The quality of the stored wastewater was improved substantially through sedimentation of particulates and microbial stabilization of organic and nutrient material.

The irrigation system was designed to irrigate 1,153 ha, 991 ha of which were irrigated by 22 electric-drive center pivot irrigation rigs. The remaining 162 hectares were irrigated by the furrow flooding technique to maximize land use in areas not accessible to the center pivot system.

Low pressure Nelson spray nozzles were used to apply the wastewater along the irrigation rigs. Each nozzle provided a 360° umbrella pattern with an effective wetted diameter of 8.5 to 9.1 m (28 to 30 ft) to allow for the greatest application intensity. The energy dissipating deflector incorporated into the nozzle assembly was a concave plastic plate. Water discharged through the orifice was deflected upward once it struck the deflector which enhanced the creation of aerosols during the period of study and increased drift and evaporation of water. Convex deflectors were installed on most nozzles after the LISS monitoring period ended (i.e., after October 1983) to direct the water downward. This change reduced aerosol formation and drift. The spray nozzles were situated on drops 3.2 m (10.5 ft) apart on 52.1 to 54.3 m (171 to 178 ft) spans between towers. Nozzle heights were 1.2 m (4 ft) to 2.1 m (7 ft) above ground, while nozzle diameters ranged from 2.4 mm (3/32 in.) up to 7.1 mm (9/32 in.) with the smaller nozzles located near the pivot and the larger ones at the end of the lateral.

A Rainbird-type end gun on each lateral could be activated to irrigate all or some of the corners. The height of the end sprinklers was from 3.0 m (10 ft) to 4.6 m (15 ft) depending upon the terrain. When the end guns were activated, their effective wetted diameter was 18.3 m (60 ft).

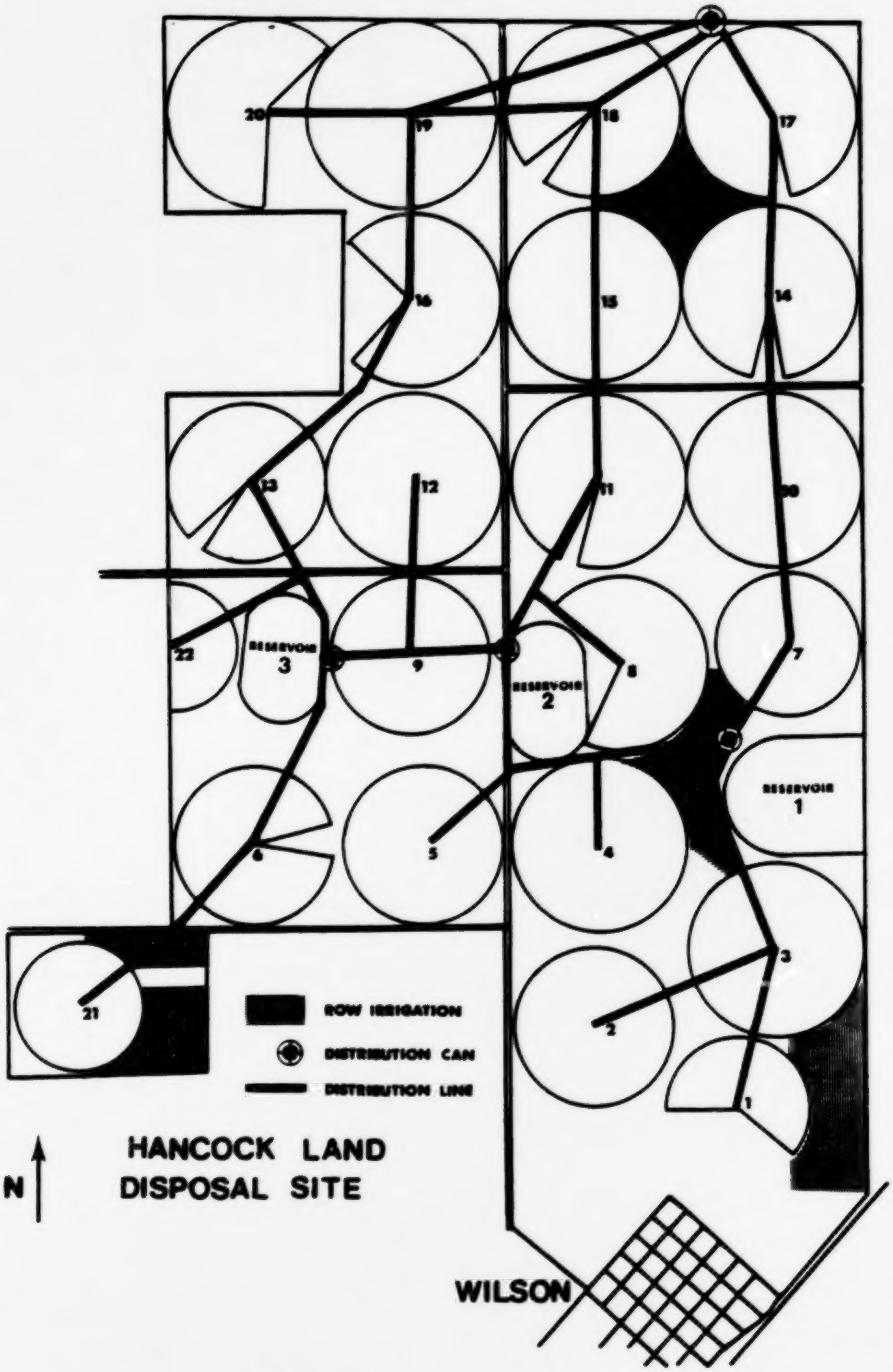
The laterals varied in length from 307 m (1007 ft) to 476 m (1562 ft) with six to eight towers per pivot. The speed of traverse of each lateral was variable, and at maximum speed a pivot could complete a full cycle in 13 or 14 hours (Sheaffer and Roland, Inc. and Engineering Enterprises, Inc., 1980).

Each center pivot was designed to irrigate up to 15 cm in 20 days after allowing for 20% loss due to evaporation. Without the use of the reservoirs, five to six center pivots could be operated at the same time, utilizing the flow pumped directly from the SeWRP. Each center pivot had a centrifugal booster pump which increased the line pressure to an operating level of 3.1×10^6 pascals (45 psi). A schematic of the Hancock farm irrigation system is presented in Figure 4.

The City of Lubbock's wastewater discharge permit required a 46-m buffer zone along the northern boundary of the farm. In addition, a 400-m buffer zone was observed immediately north of the city of Wilson. No spray irrigation was permitted within these buffer zones. Spray irrigation also was not practiced within 400 m of the homes of non-participants of the LISS. Plastic tubing measuring 3 m x 1.3 cm (9 ft x 1/2 in.) was attached to the nozzles on pivots affected by the buffer zone on the northern and western farm boundaries in order to furrow irrigate these areas, which consisted of 180 ha.

System Design and Operation in Relation to EPA Design Criteria and Recommendations

The Lubbock Land Treatment System (LLTS) was designed and operated as a large demonstration project to allow collection of research data under a wide variety of conditions. The hydraulic conveyance system from Lubbock's Southeast Water Reclamation Plant (SeWRP) to the Hancock farm was sized to accommodate a design flow of 28,000 m^3/day. The wastewater storage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4
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21
2
1
ROW IRRIGATION
DISTRIBUTION CAN
DISTRIBUTION LINE
N
HANCOCK LAND
DISPOSAL SITE
WILSON

Figure 4. Hancock farm irrigation system

and distribution system was designed to apply 66 cm (26 in.) of treated effluent per year to the Hancock farm.

Operational problems associated with wastewater management at SeWRP and odors emitted during spray irrigation with effluent transported directly from SeWRP reduced the annual flow to the Hancock farm to only 20% (4,128,000 m^3) of the total SeWRP effluent in 1982 and 19% (3,744,000 m^3) in 1983.

The City of Lubbock's wastewater discharge permit for SeWRP required the plant to produce an effluent with a 30-day-average 5-day biochemical oxygen demand (BOD_5) not greater than 45 mg/L. During the project monitoring period the effluent BOD_5 quality from SeWRP ranged from a monthly high of 260 mg/L to a monthly low of 27 mg/L:

	Average Monthly Effluent BOD_5 Produced by Lubbock SeWRP	
Month	1982 mg/L	1983 mg/L
January	143	71
February	260	120
March	198	105
April	139	65
May	108	30
June	128	39
July	130	49
August	76	27
September	69	43
October	171	31
November	63	63
December	86	49

The average fecal coliform concentration in the waste stream pumped to the center pivot irrigation machines exceeded EPA guidelines throughout the study period. The guidelines issued in November 1978 state:

> ''Biological treatment by ponds or inplant processes plus control of fecal coliform count to less than 1000 MPN/100 ml - acceptable for controlled agricultural irrigation except for human food crops to be eaten raw.'' (USEPA, 1981)

The actual flow-weighted average fecal coliform concentrations of the applied wastewater during the four major irrigation periods were:

	Fecal coliform concentration (colony forming units/100 mL)
Spring 1982	4,300,000
Summer 1982	840,000
Spring 1983	5,200
Summer 1983	120,000

A factor which affected aerosol formation and drift was the energy dissipating deflector incorporated in the spray nozzle assembly used during the research study. The deflection pad was a concave plastic plate. Since water discharged upward, the creation of aerosols was enhanced. The nozzles were replaced at the conclusion of the research study to direct the wastewater downward and reduce aerosol formation.

In summary, the LLTS expansion was designed to accommodate specific research objectives. During system operation, the fecal coliform concentration of the waste stream from SeWRP and the discharge from the storage reservoirs greatly exceeded EPA guidelines, especially in 1982. The effluent BOD_5 concentration produced by SeWRP did not satisfy Texas permit requirements until May 1983. However, the system was operated below hydraulic design capacity in 1982 and 1983.

Periods of Irrigation

Wastewater spray irrigation commenced at the Hancock farm on February 16, 1982. The infectious disease effects of irrigation occurring through September 20, 1983 were monitored by the LISS. The two major irrigation periods each year were from mid-February through April, to provide ground moisture prior to planting, and from July through mid-September, to irrigate the growing crop. The primary crops were grain sorghum, soybeans, and sunflowers in 1982 and cotton, grain sorghum, and wheat in 1983. Thus, during the 19-month interval of irrigation whose effect was observed by the LISS, there were four major periods, or seasons, of sprinkler irrigation with wastewater at the Hancock farm. Table 4 presents the dates and levels of sprinkler irrigation with wastewater from the pipeline and from the reservoirs by the 19 rigs with functional spray nozzles during these major irrigation periods, based on records maintained by LCCIWR.

TABLE 4. MAJOR IRRIGATION PERIODS AT HANCOCK FARM DURING LISS SURVEILLANCE

Irrigation period	Start date	End date	Wastewater sprinkler irrigation (cm applied[a]) from pipeline	from reservoir
''Spring 1982''	2-16-82	4-30-83	5.83	0
''Summer 1982''	7-21-82	9-17-82	6.91	3.87
''Spring 1983''	2-15-83	4-30-83	0	14.87
''Summer 1983''	6-29-83	9-20-83	0.20[b]	14.99

[a] Farm average over 19 sprinkler pivots of total centimeters of wastewater applied during irrigation period (pivots 18, 20, and 21 practicing furrow irrigation were excluded.

[b] Applied from 7-12-83 to 7-30-83.

Every center pivot rig completed at least one full circular revolution of wastewater spray irrigation in each of these four irrigation periods. The irrigation rigs generally completed a circular revolution in 2 to 5 days. Most irrigation rigs made between two and seven circular revolutions in each of these four irrigation periods. Infection events occurring in time intervals consistent with these four irrigation periods were analyzed in the LISS to investigate possible association with aerosol exposure.

In addition to these major irrigation periods, a few irrigation rigs were operated sporadically at other times, as shown in Table 5. Since the volume of wastewater applied in these irrigation events was much smaller than in the major irrigation periods, these additional irrigation events were generally ignored in the data analysis.

All of the irrigation data from Tables 4 and 5 are plotted versus time in Figure 5. The ordinate is the wastewater sprinkler irrigation rate, in centimeters per month, to adjust for varying durations of irrigation. The area of each rectangle is proportional to the volume of wastewater applied.

TABLE 5. MINOR IRRIGATION PERIODS AT HANCOCK FARM DURING LISS SURVEILLANCE

Start date	End date	Wastewater sprinkler irrigation (cm applied[a]) from pipeline	from reservoir
5-20-82	5-25-82	0.10	
10-20-82	11-18-82		0.44
12-4-82	12-16-82		0.15
5-9-83	5-12-83		0.81
5-24-83	5-28-83		0.66
6-21-83	6-24-83		0.32

[a] Farm average over 19 sprinkler pivots of total centimeters of wastewater applied during irrigation period.

The wastewater and aerosol sampling data (see Sections 5A and 5B) indicate that microorganism levels were substantially higher (by one or more orders of magnitude) in the pipeline wastewater than in the reservoir wastewater that was sprayed. Hence, it appears that the LISS population had greater aerosol exposure to most wastewater microorganisms in 1982 than in 1983. The summer 1982 irrigation appears to have been the highest period of irrigation-related exposure to many of the microorganisms studied.

B. STUDY POPULATION

Sampling

The rectangular area within 4.8 kilometers (3 miles) to the north, approximately 4.0 km to the south, and approximately 3.2 km to the east and west of the perimeter of the spray irrigation rigs on the Hancock farm was designated as the study area. This area, which includes the small city of Wilson, Texas and the rural areas north, northwest, and northeast of Wilson, was divided into six sampling zones (Figure 6). The rectangular Zone 1 included all rural households located on the Hancock farm and within approximately 0.8 km (0.5 miles) of its perimeter. Zone 2 contained the

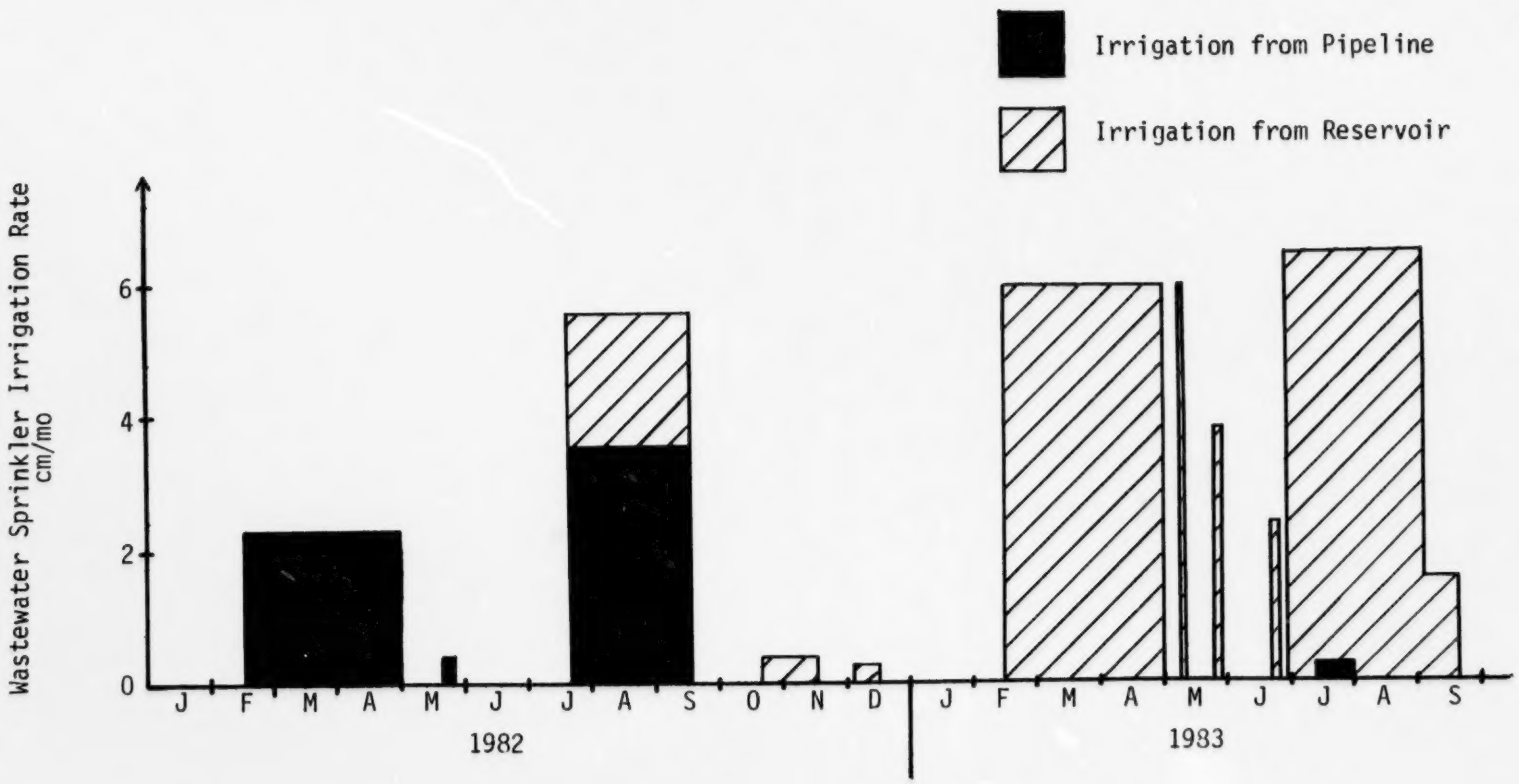


Figure 5. Wastewater sprinkler irrigation at Hancock farm during LISS

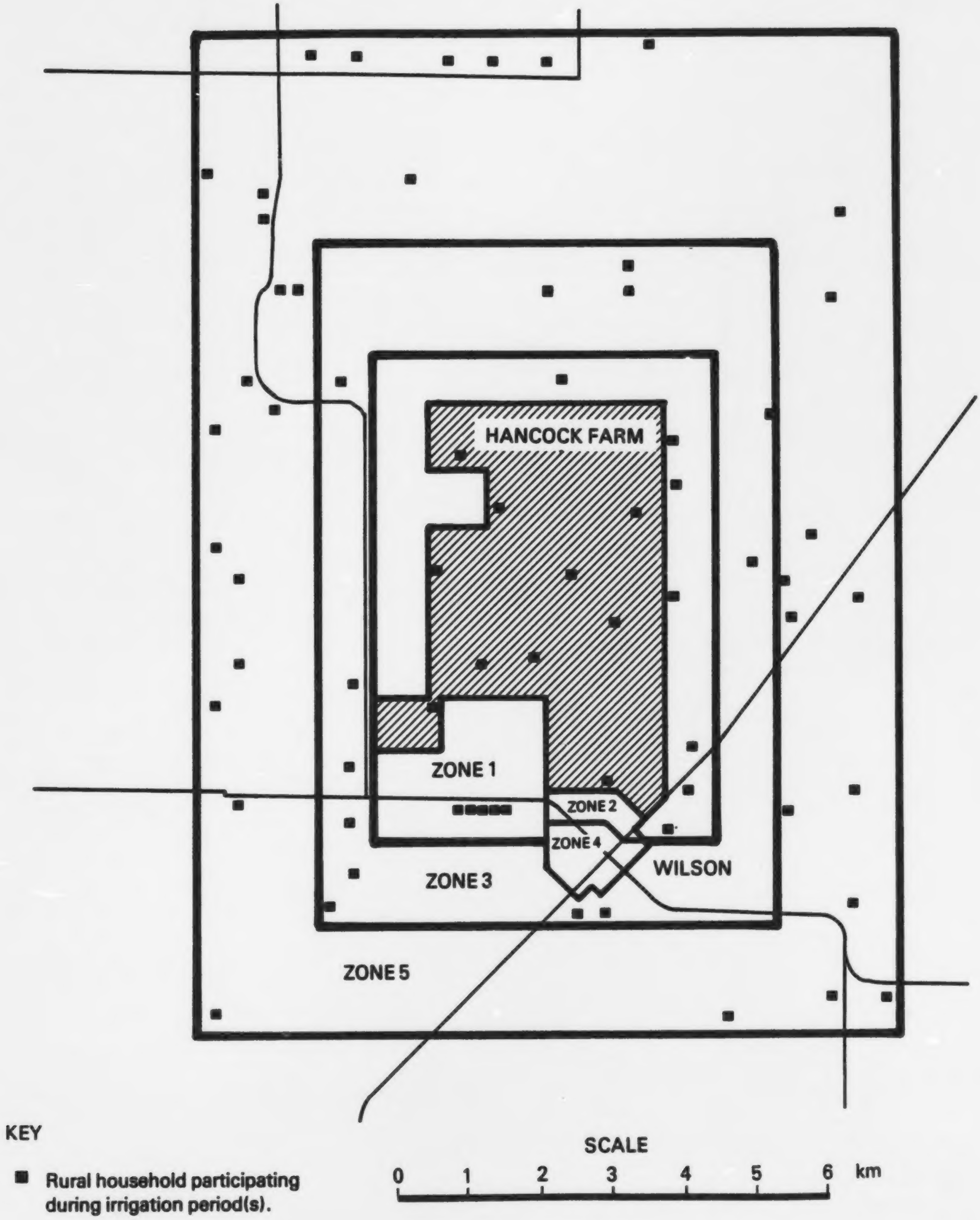

HANCOCK FARM
ZONE 1
ZONE 2
ZONE 4
ZONE 3
WILSON
ZONE 5
KEY
■ Rural household participating during irrigation period(s).
SCALE
0 1 2 3 4 5 6 km

Figure 6. Sampling zones comprising the study area

households located within 0.8 km (0.5 miles) of the Hancock site boundary within Wilson. Included in Zone 3 were all rural residences located approximately from 0.8 to 1.6 (E and W) or 2.4 (N or S) km from the Hancock farm. Zone 4 consisted of the Wilson households which were located 0.8 to 1.6 km from the site. Zone 5 contained the rural households which were approximately located from 1.6 or 2.4 to 3.2 km (E and W), 4.0 km (S) and 4.8 km (N) of the Hancock farm boundary. Zone 5 was extended to approximately 4.8 km north of the farm due to the prevailing southerly winds. The households of the small number of Hancock farm workers who resided outside the study area were placed in Zone 6. The size of the sampling zones had no impact on the LISS results, since all data analyses were based on an aerosol exposure index rather than sampling zone.

Due to the limited number of residences in the rural area (approximately 130), all households within Zones 1, 3, 5, and 6 were invited to participate in the study. Special emphasis was placed on recruiting all households located in Zone 1 in order to maximize the amount of information from individuals who, presumably, would be most highly exposed to wastewater aerosols.

There were approximately 172 households located within Wilson, and one-half of these were selected for recruitment into the study. Thus, every other Wilson household was designated a part of the sample. When a refusal was obtained, the next available house on the block was contacted, according to a standardized selection procedure.

Households which dropped from the study before June 1982 were replaced with households in the same sampling zone whenever possible. The study population is not considered to be transient; however, several households did relocate within the study area and many individuals temporarily moved out of the study area. In these cases, the affected households and individuals were asked to continue their participation in the study as long as they were residing within the boundaries of the study.

One hundred ninety seven households with 580 members were recruited into the study. Thirty-four of the households (102 members) which were recruited in May 1980 never actually participated in the study. One hundred sixty three households, with 478 members, participated at some level during the course of the study. One hundred seven (66%) participating households with 306 (64%) participants remained in the study until its conclusion in October 1983. Twenty-four percent of the participants dropped out of the study between June 1980 and January 1982, prior to the onset of irrigation. Only 12% of the participants dropped out of the study after irrigation had commenced.

Health Interview and Recruitment

A team of interviewer-recruiters was trained and obtained the medical history of each family member in the sample households. Each interviewer received an instruction manual describing methods for conducting the interview and recording illness history. Interviewers were instructed in methods of recruiting residents to participate, in maintaining health diaries, in submitting to tuberculin testing, and in providing stool, illness, and blood specimens. The purpose, duration, and incentives for participation in the study were explained to each interviewer to enable him/her to respond to questions from interviewees during the recruitment period. The incentives included: 1) continuing information about the health status of each participant, 2) laboratory information regarding infectious agents recovered from specimens collected during an illness, 3) a brief layman's version of the findings from the study, 4) a small monetary reward at the end of each study year for the inconvenience imposed on each participant for cooperating in the health watch, and 5) small payments for each fecal specimen provided.

A questionnaire was developed to record information on the number of members in each family, their age, level of education, occupation, income, chronic health conditions, and relevant medications. This form is presented in Appendix B. A pretest of the instrument was done to evaluate the interviewee's understanding of and responses to the questions being asked. The interview required 15 minutes of participant time.

Update questionnaires were administered over the telephone to all participating households in February 1982 and October 1983. These questionnaires are presented in Appendices C and D. These questionnaires were designed to document changes (in chronic health conditions, occupation, use of air conditioning etc.) during the course of the study. The questionnaire updates were also used to obtain needed additional information, such as the polio immunization history of children, the type of air conditioning system, degree of water consumption, and frequency of contact with large groups.

Serosurvey

Twice each year during the study (usually during June and December), each participant was contacted (by mail and telephone) and asked to provide a blood sample at the Wilson Community Center. Blood was collected by venipuncture into two sterile 15 mL serum separation vacutainers. Syringes (10 mL) were used to collect blood from children who were under the age of two.

Blood specimens were placed on ice and shipped to the serology laboratory (UTSA in 1980-1981; UI in 1982 and 1983) for serum separation and storage. Upon arrival at the laboratory, the serum was separated from the clot, dispensed into four (UTSA) or five (UI) vials, and catalogued. All but one vial were stored at -70°C. The remaining (UI) vial was heat-inactivated and stored at -20°C for use in enterovirus serology.

Allowing for variations between participants, approximately 7 - 8 mL of serum was obtained from each participant. The serum was divided into five aliquots: two aliquots were allocated for immediate testing (serum neutralization, *Legionella*, reovirus, and rotavirus); one aliquot was reserved for hepatitis A serology (at either UTSA or Metpath); one aliquot was used for ''special testing'' (Norwalk virus, *E. histolytica*) at other laboratories; and, the final 1 mL aliquot was stored and later forwarded to the archive at EPA HERL. In cases where only small volumes of blood could be collected from a small child or from a participant with collapsed veins, the archived specimen was aliquoted first, and the remaining serum was allocated for as many tests as possible. In cases of severe shortages, children's sera were reserved for Norwalk virus and rotavirus serology.

Informed and parental consent forms (Appendix E) were signed prior to collection of the first blood sample from each participant. Consent forms were updated and administered for a second time in June 1983.

Every effort was made to obtain a blood sample from each person in every participating household. Participants who could not, or did not, come to the regularly scheduled blood drawing clinics were contacted by phone and asked to provide a blood sample in their home or at a follow-up clinic. These follow-up measures increased the overall number of samples collected by 10 to 30%.

Fecal Specimens

During 1980 and 1981, regularly scheduled fecal specimens were requested for children age 12 and under. In cases where the household had only one child in the age group, the next oldest household member was also recruited as a donor. Due to the fact that only one of three eligible households on the Hancock farm and two of five eligible households in Zone 1 regularly provided specimens in 1981, one randomly selected adult from every study household was asked to provide a specimen in 1982. If the selected adult was not willing to provide a specimen, then another family member was asked to provide a specimen for the household. In households that provided specimens in 1980 and 1981, the same members were asked to continue providing specimens in 1982. Only two specimens per household were accepted in 1983 in order to limit the number of specimens received to 100 per collection period.

Collection of the children's specimens took place over three 2-week periods in 1980 and six 2-week periods in 1981 (see Figure 1.C.1). In 1982, each of the six collection sessions took place over a 1-week period that was coordinated with the irrigation schedule. Collections took place over five 1-week periods in 1983. In order to obtain a maximum amount of information during periods of irrigation in 1982 and 1983, three consecutive specimens were solicited. One sample was collected prior to the onset of irrigation and the remaining two samples were collected during the irrigation period. A $5 fee was offered for each specimen and a $15 bonus was paid to each participant who provided the three consecutive specimens.

The Sage stool specimen system was used to collect the fecal specimens. Each household was provided with a collection kit, a styrofoam ice chest, and an ice pack. Participants were instructed to keep the specimen cold until it could be presented at the collection point in the Wilson Community Center. Participants were also asked to submit specimens as quickly as possible after collection. In cases where it was not possible or convenient for participants to bring the specimens to Wilson, a telephone number was provided to participants so that arrangements could be made for a staff member to transport the specimen.

The fecal specimens were processed by transferring approximately 10 grams to each of two appropriately labeled sterile containers. Ten mL of phosphate-glycerol buffer (pH 7) were added to one container to preserve bacterial viability. The other container was shipped without addition of any preservative. Processed specimens were stored on wet ice and shipped in biomailers with icepacks. Most specimens arrived at the UTSA Laboratory (and the UTA laboratory in 1983) within 24 to 36 hours after actual specimen collection.

All study participants were asked to provide a specimen for ova and parasite analysis in conjunction with the regular specimen collections during the summer of 1983. A subject fee of $5 per specimen was offered for each of these specimens.

Specimens for ova and parasite analysis were preserved in vials containing formalin (5% solution) and polyvinyl chloride. All materials for preservation and shipping were provided by the Texas Department of Health (TDoH). The preserved specimens were held at room temperature until the end of each collection week, then shipped to the TDoH laboratory in Austin for analysis.

Illness and Exposure Monitoring

Participating households were asked to record and report information for any of the following conditions for each participating household member:

- all acute illnesses;
- contacts with wastewater (and aerosols in 1983);
- absences of 2 days or more from the study area.

All diary information was collected by the field representatives and forwarded to UI on a biweekly basis for review and coding. The coding information was then forwarded to the Data Management section for data entry. Diary data were collected from the entire population in the summer of 1980, spring and summer of 1981, and January through October 1982. Information was collected from approximately half of the population between November 1982 and October 1983. Methods for collecting the diary information were modified several times during the course of the study in order to improve the quality of the data and to minimize the amount of time that was needed to process the information.

During 1980, each household was provided with a booklet (Appendix F) to record all illness events. At the end of each 2-week data collection period (DCP), a field representative collected the booklet and gave the household a new booklet for the next DCP. This procedure had been used successfully in a previous study (Carnow et al., 1979); however, the results were less than satisfactory in the present study. The problems that were encountered with the 1980 health diaries included:

- Since participants frequently were not at home, field representatives had to make several trips over a 2- or 3-week period in order to retrieve a diary from a single household.

- Since participants frequently forgot to complete the diary until the field representative arrived to collect the diary, the information was often based on recall of events that may have occured 2 or more weeks prior to the arrival of the field representative.

- Participants' entries in the diaries often were incomplete and inconsistent.
- Some hispanic families reported no illnesses because the adults in the household could not understand the written instructions or could not write in English.

In 1981, field representatives contacted each household by phone on a weekly basis. The field representatives were instructed to ask a series of questions and record the responses on a health diary form. The completed forms were mailed to UI after each DCP. The procedure modifications improved the quality and consistency of the illness information. However, contact with some households continued to be a problem. Field representatives frequently would try to obtain illness information 3 weeks after the week in question, resulting in data which was based on recall of events which could have occurred weeks earlier. It also caused the field representatives to mail the diary forms to UI a month to 2 months after the end of the DCP in question. Therefore, the review and coding processes were delayed, and Data Management received the coded materials several months after the illness events occurred.

The household diary form was modified in 1982 to include questions about contact with wastewater (see Appendix G). Procedures for collecting the information were also modified to correct the problems that were experienced in 1981. The change in procedures allowed illness information to be collected and analyzed quickly so that illness surveillance could be maintained for the study population during periods of wastewater irrigation. Field representatives were instructed to attempt to contact all households by phone within 2 days after the DCP had ended. At the end of this 2-day period, the field representatives transmitted the following information to UI by phone:

- study participants who reported an illness;
- type of illness;
- dates of onset and conclusion of illness;
- households that could not be contacted;
- study participants who were out of town for 2 or more days during the week.

The UI staff made an additional attempt to reach the uncontacted households and then used the information to compile a weekly summary. The weekly summary (Appendix G) listed the number of participants who were contacted and the number of new illnesses (by type) that were reported. All illnesses reported in sampling Zone 1 (Hancock farm families and rural households within one-half mile of the farm) were also noted in this report. This provided a rapid method for comparing illness rates of participants who lived in the high exposure zone to the illness rates for all study participants. The weekly summary was distributed to all concerned investigators within 4 days after the week of interest had ended.

Illness information was also reviewed on a weekly basis to determine if any unusual patterns of illness had developed. Patterns of interest included geographic distribution of illnesses, age distribution of illnesses, unexpected increases in respiratory or GI illnesses, and unexpected reoccurrences of illness in high exposure households.

Beginning on October 24, 1982, the number of families contacted on a weekly basis was reduced by approximately half. The distribution of households which were included as ''sentinel families'' is listed by sampling zone in Table 6. All households with members who had exposure to wastewater were included on the sentinel family list. The remainder of the families were selected on the basis of geographic distribution, family size, and the family's past record of participation.

TABLE 6. COMPARISON OF SENTINEL POPULATION TO STUDY POPULATION IN OCTOBER 1982

Zone		Study population in Oct. 1982				Sentinel population			
		Households	Adults	Children	Total	Households	Adults	Children	Total
Rural	1	22	37	13	50	22	37	13	50
	3	9	20	6	26	6	12	3	15
	5	31	61	30	91	12	23	15	38
Wilson	2	33	57	37	94	11	19	11	30
	4	33	55	35	90	13	25	16	41
	6	4	4	3	7	2	4	3	7
Total		132	234	124	358	66	120	61	181

The weekly diaries were modified again in 1983 to obtain more complete information about direct contacts with wastewater and to include weekly information about the sentinel family's exposure to wastewater aerosols. The modified diary form and exposure questionnaire are included in Appendix G. Prior to implementation, a draft form of the exposure questionnaire was submitted to selected study participants and staff members for comments and suggestions. Comments were used to revise the format and the new questionnaire was implemented in conjuction with the onset of irrigation in February 1983.

Illness Specimens

Field representatives were instructed to request permission to collect an illness specimen from a study participant whenever the participant reported the recent onset of an illness. Throat swabs were collected within a 3-day period after a participant reported the onset of a respiratory illness. Stool specimens were collected within a 10-day period after a participant reported the onset of GI or respiratory symptoms. Study participants were also actively encouraged to contact the field representatives immediately after the onset of a respiratory or GI illness to request that illness specimens be collected.

The procedure for collection of throat swabs was taught to the field representatives by personnel at the Texas Department of Health. The Marion Culturette II swabs were used for collection and preservation. In most instances, two swabs were used for each illness specimen.

An illness specimen was labeled ''acute'' if collected while the participant was displaying symptoms of the illness. A specimen obtained within 1 week after the participant had recovered from symptoms of the illness was termed a convalescent illness specimen. A follow-up specimen sought to clarify the etiology of an unusual finding was labeled as ''requested'' specimen. All specimens were kept on wet ice and shipped to UTSA laboratories as quickly as possible. Abnormal results were promptly reported to the participants.

Activity Diaries

Each participating household was provided with an activity diary form and a map during four 1-week periods in 1982 (in March, April, August and December) and two 1-week periods during 1983 (in April and July). In addition, Hancock farm residents were asked to provide two additional diaries in March and August 1983. Participants were asked to use the diaries to record the amount of time that they spent in each of the designated areas on the map. They were also asked to record the amount of time that they spent at home and in Lubbock. This diary information was used to develop a wastewater aerosol exposure index for each participant during each of the four irrigation seasons.

The activity diaries which were sent to the households in March and April were returned to UI in the self-addressed, stamped envelopes which were mailed with the diaries. Due to the low compliance rate (55% in March, 41% in April) and the high number of incorrectly completed diaries, subsequent activity diary periods were scheduled to coincide with a fecal collection or a blood drawing. This scheduling allowed the health watch manager or the field representatives to be available to help participants correctly complete the diaries. It also allowed follow-up in cases where participants did not respond to the request for activity diaries. This modification resulted in an 80 to 90% response rate which was a marked improvement. Previous activity diaries were used in cases where the participant indicated that his activities had not changed since the previous recording period. The diary form and the maps for the irrigation seasons have been included in Appendix H.

Tuberculin Skin Testing

Tuberculin skin tests were performed in order to monitor possible nontuberculosis mycobacterial (NTM) infections. These tests were administered in June or December 1980, June 1981, December 1982, and October 1983. The interdermal Mantoux test (5 TU of PPD-S injected intracutaneously into the volar surface of the forearm) was performed by Texas Department of

Health nurses. All participants were asked to report back to the Wilson Community Center within 48 to 72 hours after the test was administered. The public health nurses or the health watch manager examined all cases with erythema and measured all indurations. Indurations which were found to be 10 mm or greater were referred to the Health Department.

Poliovirus Immunization

Based on serological analysis of the first blood sample collected, a significant proportion of the study population appeared to be susceptible to at least one of the three poliovirus serotypes. Because poliovirus was found in the Lubbock effluent, prophylactic immunization of susceptible residents (particularly those within 400 m of a spray rig) was recommended and implemented.

All participants who gave a blood sample were notified by mail or telephone of their poliovirus immune status and as to whether immunization was recommended. (A susceptible individual was defined as someone who had a serum titer of less than 8 against one or more of the poliovirus serotypes by serum neutralization. Individuals with titers greater than 4 for all three serotypes were considered immune.)

Special immunization clinics were conducted at the Wilson City Hall by the Texas Department of Health, and all susceptible participants were invited to attend. The first clinic was held in early April 1981 in order to allow time for immunity to develop before the initiation of irrigation. Subsequent clinics were conducted in May and June and in January 1982. Study participants could also receive immunization at the Health Department clinics in Lubbock or Tahoka if they preferred.

In accord with the Texas Department of Health's recommendations, susceptible adults (18 years or over) received four doses of the Salk inactivated polio vaccine (IPV). Injections were given monthly from April through June 1981, and a booster shot was administered in January 1982. All susceptible children received the Sabin oral vaccine (OPV) booster dose in May 1981.

All individuals submitting to the imunization signed the informed consent form which is used by the Health Department. (Parents signed for minors.) A copy of this form is presented in Appendix E. All individuals attending the clinic also received a short polio immunization history questionnaire. The questionnaire was administered by the field representatives by telephone to individuals who did not attend the clinic. When an individual was deemed susceptible by serological analysis but presented proof of immunization, a booster immunization was recommended.

A summary of the poliovirus protection status of participants is listed in Table 7.

TABLE 7. SUMMARY OF PARTICIPANT POLIOVIRUS PROTECTION STATUS (January 1983)

Study populations	Children	Adults	Total
Total number tested	158	274	432
Number recommended for immunization	71	123	194
Number receiving complete immunization series	63[a]	61	124
Number receiving incomplete immunization series	0	46	46
Number refusing immunization	8	16	24
Number current study participants who have not given blood	10	8	18

[a] All children who were recommended for immunization had a previous history of immunization. Therefore, only a booster dose was administered.

Restaurant Patronage Survey

To investigate food preparation as a possible source of the bacterial infections observed in 1982 and 1983, a restaurant survey was administered retrospectively by telephone to all available fecal and illness specimen donors in July 1984. Although the primary intent was to determine how frequently participants ate food which was prepared at one restaurant during the summer of 1982, the restaurant survey was designed to include all four establishments which served food in Wilson during 1982 and 1983. Only two of the establishments, restaurants A and B, were open for business during the entire 1982-1983 period of time. The other two establishments were actually small grocery stores which prepared food (mainly sandwiches) as a sideline. Restaurant B was the only establishment which served food that could be eaten on the premises; the other establishments prepared food on a take-out basis only.

It was anticipated that participants would not remember exactly how many times they had eaten food prepared by a restaurant 2 years earlier. Therefore, respondents were asked to estimate how often, if ever, the specimen donor was likely to have eaten food prepared by each of the restaurants during the summers (i.e. June-August) of 1982 and 1983. The choices offered to the respondent were:

- more than once a week;
- once a week to once a month;
- less than once a month;
- never.

The respondent was also asked to compare the frequency of patronage in summer to patronage of the restaurant during the rest of the year. The survey questionnaire is presented in Appendix I. Since this was a small rural community, most respondents had no difficulty with recall or knowledge of the donor's patronage frequency.

C. EXPOSURE ESTIMATION

Aerosol Exposure Index (AEI)

A measure was needed of the degree of a participant's cumulative exposure to microorganisms in the wastewater aerosol, relative to all other study participants during a given irrigation period. The aerosol exposure index, AEI, was used in all LISS data analyses as this measure of relative exposure to the wastewater aerosol.

AEI was constructed using a microenvironment approach to estimate the cumulative relative exposure of each participant to the pathogens in the wastewater aerosols sprayed during each irrigation period. Estimated aerosol exposures due both to distant transport of aerosols and to extensive contacts with the aerosol mist and at short distances downwind from an irrigating rig were accumulated in AEI as a weighted sum:

$$\mathrm{AEI} = \mathrm{EI} + 0.5 \cdot \mathrm{XAEREM}$$

EI, the aerosol transport exposure index, was based on activity diary data and on dispersion modeling of historical wind data for five microenvironments, as discussed below. XAEREM, the index of extensive aerosol exposures, was based on an exposure log which the sentinel participants provided throughout the 1983 irrigation period for the downwind aerosol plume microenvironment. The definition of XAEREM is presented in the next section, Additional Exposure Measures. EI and XAEREM exhibited similar highly skewed distributions, but XAEREM was much larger than EI for the small number of participants with occupational exposure to the wastewater and its aerosol mist. The coefficients of 1.0 for EI and 0.5 for XAEREM were chosen empirically to yield an intuitively reasonable ordering of AEI among participants: the contribution to AEI from documented extensive contacts with the aerosol mist should dominate the contribution from inferred distant transport of aerosols. It should be noted that the aerosol densities sampled in the LISS (see Section 5B) were not used in the calculation of AEI.

For each participant during each of the four periods of irrigation, a value of EI was computed from activity diary data and dispersion modeling of historical wind data for five microenvironments (h and i = 1, 2, 3, 4) as:

$$\mathrm{EI} = \left(P_h T_h + \sum_{i=1}^{4} \bar{P}_i T_i\right) (S + 1)/2$$

where h - household location
i=1 - blue activity diary map area (Hancock farm)
i=2 - orange activity diary map area (surrounding Hancock farm)
i=3 - white activity diary map area (remainder of study area)
i=4 - outside study area

T_h - weighted average of hours that the participant is at home during the applicable weeks of the activity diaries
T_i - weighted average of hours that the participant is in microenvironment i (i=1,4) excluding hours at home during the applicable weeks of activity diaries ($T_h + \Sigma T_i = 168$)
P_h - predicted relative aerosol concentration at the participant's home
$\bar{P}_i$ - average predicted relative aerosol concentration in microenvironment i (i=1,4) calculated as the geometric mean of the P_h values for all study households in the microenvironment
S - proportion of days during the irrigation period that the participant is reported to be in the study area (from the weekly health report)

As the product of estimated relative microorganism concentration in the air of a given microenvironment and his time spent in that microenvironment accumulated over all microenvironments in the study area, EI provided a crude estimate of a given participant's cumulative inhaled dose due to aerosol transport by the wind, relative to all other participants during that irrigation period.

The predicted relative aerosol concentration of microorganisms at a given distance d from the edge of the nearest irrigation rig on the Hancock farm was estimated according to standard dispersion modeling concepts as

$$P_d = D_d e^{\lambda d/\bar{u}} = D_d e^{(-0.005\ \mathrm{sec}^{-1})d/(5.0\ \mathrm{m/sec})} = D_d e^{-0.001\ d}$$

where d - distance in meters from edge of nearest irrigation rig on Hancock farm
$\lambda = -0.005\ \mathrm{sec}^{-1}$ - median decay rate of aerosolized wastewater microorganisms determined in Pleasanton, CA study (Camann, 1980)
$\bar{u}$ - average wind speed = 5.0 m/sec for Lubbock (1965-74)
D_d - normalized aerosol concentration at point d resulting from diffusion, based on 1965-74 wind patterns for Lubbock for the months of the irrigation period

The normalized aerosol concentration D_d due to diffusion (i.e., assuming no microorganism die-off) was estimated for each irrigation period using a time-averaged dispersion model computer program. Model inputs included wind speed and wind direction data stratified by stability category and source emission rates. Source emission rates of the rigs were calculated assuming uniform areal application so each emission rate was proportional to the area sprayed by the rig. Rigs with dropped lines were assumed to produce no aerosol. D_d had to be calculated prior to the start of irrigation so activity diary maps defining the microenvironments could be constructed and used during irrigation to obtain the participant time estimates T. Consequently 10 years (1965-74) of historical wind data from the Lubbock airport were used to calculate D_d isopleths separately for the February-April and the July-August time periods.

Wind roses from the actual periods of irrigation (see Figures A-3 to A-6 in Appendix A) were later compared to 5 years of the historical data (see Figures A-1 and A-2 in Appendix A) to ascertain the validity of using the historical data. There were some differences from one year to another in the distribution of wind directions during the ''spring'' irrigation period (mid-February through April). While the primary wind direction remained from south-southeast through southwest, the secondary wind direction for this irrigation period was from the northwest in 1983, whereas it was from the north through east both in 1982 and in the 1969-73 period. In contrast, the wind direction distributions during the summer irrigation periods of 1983, 1982 and 1969-73 were very similar. Hence the EI values for the spring 1983 irrigation are probably somewhat less accurate than for the other three irrigation periods. However, the magnitude of this effect is relatively small considering the manner in which AEI is used in the LISS.

An exponential decay factor was multiplied by D_d to estimate the relative microorganism aerosol concentration P_d. Decay rates λ have been observed to be highly variable both among microorganism groups and for the same microorganism group under different environmental conditions. A decay rate of $\lambda=-0.005\ \mathrm{sec}^{-1}$ was assumed. This value was both the median and the slowest detectable rate of various microorganism groups obtained for the sprinkler wastewater aerosol at Pleasanton, CA (Camann, 1980).

The maps used with the activity diary to define the microenvironments for time reporting (T_1, T_2 and T_3) are presented in Appendix H. Microenvironment i=1 of highest exposure was colored blue on the maps and consisted of the Hancock farm (excluding the 400-meter buffer area north of Wilson). The boundary between microenvironment i=2 of less exposure (colored orange) and microenvironment i=3 of still lower exposure (left white) was chosen utilizing a D_d diffusion isopleth modified slightly for landmarks recognizable by participants and for microorganism die-off. The map used with activity diaries collected during the school year (in March, April and December) was based on a D_d isopleth from the historical February-April wind data since that was the primary period of irrigation during these months. The summer activity diary map used a D_d isopleth from the historical July-August wind data.

Exposure index estimates were computed for each of the four major irrigation periods:

1. Spring 1982: Feb 16-Apr 30, 1982
2. Summer 1982: Jul 21-Sep 17, 1982
3. Spring 1983: Feb 15-Apr 30, 1983
4. Summer 1983: Jun 29-Sep 20, 1983

The values obtained for P_h and the $\bar{P}_i$ in each irrigation period are summarized in Table A-1 in Appendix A.

Participants were asked to keep activity diaries during six selected weeks as a means to estimate their T_h and T_i values during each irrigation period. A sample activity diary is presented in Appendix H. The six weeks in which activity diaries were kept (see Figure 7) were in data collection periods (DCP) 206 (March 21-27, 1982), 208 (April 20-26, 1982), 216 (August 1-7, 1982), 224 (November 28-December 4, 1982), 308 (April 10-16, 1983), and 314 (July 10-16, 1983).

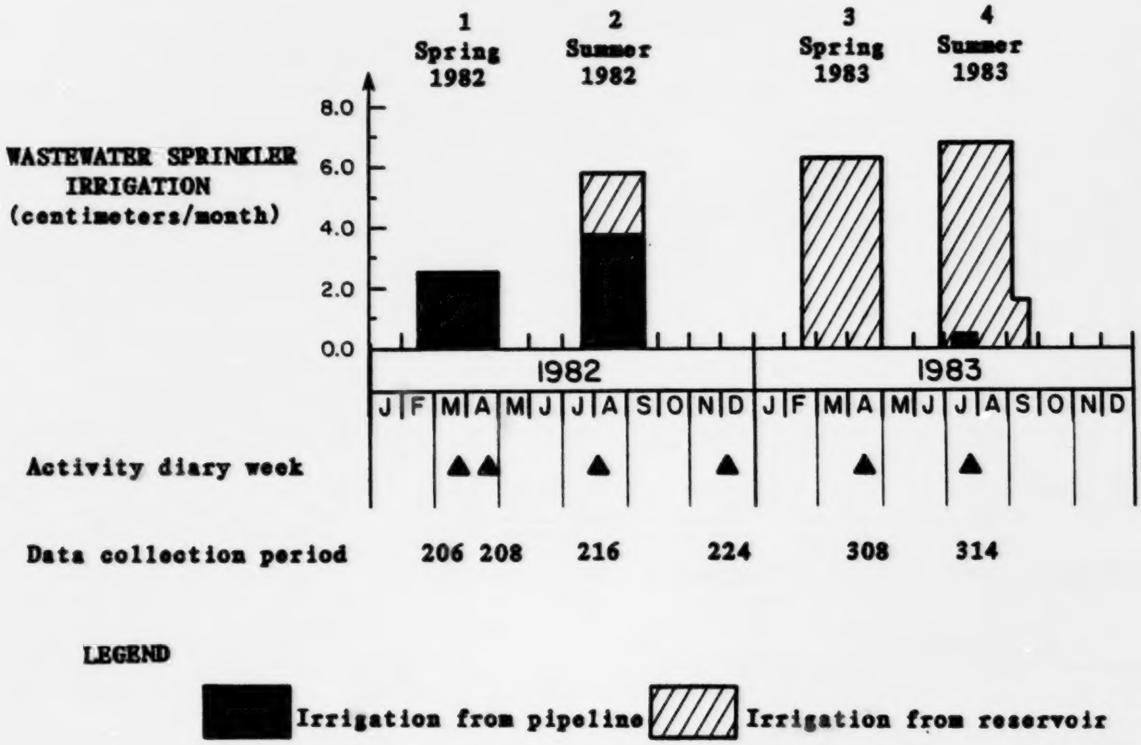


Figure 7. Relation of activity diary collection weeks to major periods of irrigation

In the last three activity diary periods (i.e., DCPs 224, 308 and 314), participants whose activity pattern was basically unchanged and who spent little time in the vicinity of the Hancock farm were allowed to certify that the activity information provided in a prior activity diary was applicable. In these cases, the activity information from the applicable prior activity diary was substituted.

A weighted average of the time reports from the applicable activity diaries was employed to estimate T_h, T_1, T_2 and T_3 for each irrigation period. Full weight was given to activities diaries concurrent with the irrigation period, half-weight to diaries from the same season of the other year, and quarter weight to other diaries during the same school year. The resulting weighted averages were:

1. Spring 1982: $T = (2T_{206} + 2T_{208} + T_{308})/5$
2. Summer 1982: $T = (2T_{216} + T_{314})/3$
3. Spring 1983: $T = (4T_{308} + 2T_{206} + 2T_{208} + T_{224})/9$
4. Summer 1983: $T = (2T_{314} + T_{216})/3$

Missing activity diaries were excluded in calculating the weighted average.

Cases in which the T_h and T_1 time reports from the lesser weighted activity diaries differed substantially from the concurrent activity diaries were evaluated to determine whether all the data were applicable to the weighted average. The reported activity data were checked for errors when 1) the times at home T_h reported on the activity diaries from the same season differed by more than a factor of 2, or 2) the times spent on the Hancock farm T_1 reported on the activity diaries from the same season differed by more than a factor of 10. The participant's T value from the most similar season was substituted if none of the activity diaries in the weighted average were provided. If a participant provided none of the six activity diaries, his T values were estimated based on the best available knowledge of his usual major activities.

When participants were not home during the majority of the activity diary collection week, they were asked to complete the activity diary for the week of their return. Hence, a downward adjustment factor (S+1)/2 to the T values were included in the EI calculation to reduce cumulative exposure for days during the irrigation period when the participant was away from the study area.

The relative precision of a participant's AEI estimates was dependent on his degree of compliance in providing activity diaries and exposure logs. Accordingly, a quality code was assigned to each AEI estimate based on the degree of reporting the applicable activity information.

Additional Exposure Measures

Other exposure measures were developed to investigate alternative routes of exposure to wastewater irrigation besides the wastewater aerosol. Each sentinel participant was asked to maintain a log of extensive wastewater contacts from February through September 1983. As part of the weekly illness report, the most extensive aerosol exposure and direct wastewater contact of the week and the estimated hours spent on the Hancock farm were also obtained for each household member. From these data, cumulative measures of extensive aerosol exposure (XAEREM) and direct wastewater contact (XDIREM) were calculated using the microenvironment method for each sentinel participant for both of the irrigation periods in 1983. The hours spent on the Hancock farm were also averaged as another exposure measure (FHRSEM).

If a sentinel participant received exposure to the mist or aerosol from an operating spray rig within 400 yards downwind at least once during week j, the downwind distance category d(j) and duration category m(j) of the most extensive aerosol exposure were reported. The index of extensive aerosol exposures, XAEREM, was calculated as the average of these exposures for the n weeks comprising an irrigation period:

$$\text{XAEREM} = \frac{1}{n} \sum_{j=1}^{n} c_{d(j)} \cdot u_{m(j)}$$

The distance category was converted to an aerosol concentration $c_{d(j)}$ based on the geometric mean aerosol concentration of fecal streptococci from the 15 aerosol runs conducted downwind of irrigation rigs which sprayed wastewater received directly from the pipeline:

Downwind distance, d(j)	c_d
<50 yards	120 cfu/m^3
50-200 yards	18 cfu/m^3
200-400 yards	1.6 cfu/m^3

Fecal streptococci were chosen because they are hardy (Camann, 1980) and may thus serve as a useful model for enteroviruses. The pipeline runs were chosen because they provided usable data out to 400 yards. The duration category m(j) was converted to an assumed duration $u_{m(j)}$ considerably less than the midrange because of the presumed skewness of the duration data:

Duration, m(j)	u_m
<0.5 hr	0.2 hr
0.5-4 hr	1.0 hr
4-12 hr	6 hr
>12 hr	15 hr

Certain individuals who resided near an operating spray rig neglected to report aerosol exposures received while at home. From available data on the dates of operation of nearby irrigation rigs, on wind direction and on time spent at home, these aerosol exposures at home were estimated. The estimates were included in the XAEREM calculation for weeks in which aerosol exposure reports were evidently lacking.

If a sentinel participant had direct contact with the wastewater at least once during week j, the degree category k(j) and the duration category l(j) of the most extensive direct contact event were reported. The index of extensive direct contacts with wastewater, XDIREM, was calculated as the average of these contacts for the n weeks comprising an irrigation period:

$$\text{XDIREM} = \frac{1}{n} \sum_{j=1}^{n} w_{k(j)} \cdot t_{l(j)}$$

The degree of contact was converted to a numerical measure of presumed severity $w_{k(j)}$:

Degree of contact, k(j)	$w_{k(j)}$
on clothing and/or shoes	1
on skin and/or hair	10
in eyes and/or mouth	100

The duration category was converted to an assumed duration $t_{l(j)}$:

Duration of contact, l(j)	$t_{l(j)}$
<5 min	1 min
5-60 min	10 min
>60 min	100 min

The average hours per week spent on the Hancock farm, FHRSEM, was calculated from the weekly reports for nonresidents of the farm and from additional activity diaries provided by the farm residents. The weighted average T_1 from activity diaries was used as the FHRSEM value for participants who did not provide the weekly exposure log data.

Every participant with any anticipated exposure to wastewater piped from Lubbock was followed as a sentinel participant. Thus, XAEREM and XDIREM were set to zero for every nonsentinel participant since extensive wastewater exposures were assumed to be very unlikely for them.

Values of the additional exposure measures and levels for the spring 1982 and summer 1982 irrigation periods were inferred from the corresponding 1983 values except when the participant's activity pattern had changed. In particular, the XAEREM values for the spring and summer 1982 used in the AEI calculation for these irrigation periods were the XAEREM values for corresponding 1983 irrigation season, except for the 14 participants whose activity patterns had changed. Presumed XAEREM values were substituted in these cases, based on knowledge of their activities on the Hancock farm.

D. ENVIRONMENTAL SAMPLING

Wastewater

Samples of Lubbock wastewater were collected from three locations:

- the effluent from Trickling Filter Plant 2 (LTFP) at the Lubbock Southeast Water Reclamation Plant (from June 1980 until February 1982 when the pipeline to the Hancock farm became operational)
- pipeline effluent at the Hancock farm (from February 1982 to September 1983)
- effluent from the storage reservoirs at the Hancock farm (from June 1982 to September 1983)

Concurrent samples of Wilson wastewater were also collected from June 1980 to September 1983. The dates of sample collection and the types of microbiological assays performed on each sample are given in Tables A-2, A-3 and A-4 in Appendix A. The time series of microorganism concentration data from each sample location characterized each wastewater source. An overview of the frequency of measurement in the sprayed wastewater and the Wilson wastewater of each infectious agent monitored in the study population was presented in Table 2.

Twenty-four-hour composite samples of the LTFP effluent were obtained. In 1980, six consecutive 4-hour time-weighted samples of effluent were collected with an ISCO Model 1580 automatic sampler. A flow-weighted composite sample was prepared based on plant flow data for each 4-hour period. During collection each 4-hour sample was cooled at 4°C, and after compositing the final large volume sample was transferred to sterile bottles and shipped in a 4°C environment to the UTSA-CART laboratories via either airline parcel or bus express service for analysis within 24 hours. A complete description of equipment used, sampling procedure, and compositing calculation are shown in Appendix J. After 1980, three 8-hour samples were collected and flow-weighted to prepare all 24-hour composite samples.

A pipeline effluent sample at the Hancock farm replaced the sampling location previously used at the LTFP when the pipeline became operational in February 1982. Compositing for the 24-hour pipeline sample was accomplished by a time-weighting method rather than the flow-weighting method previously used due to the expectation that flows in the pipeline would be more uniform than the effluent flows experienced at the LTFP.

The pipeline was sampled at Distribution Can 4 at the end of the 19-mile pipeline and just before distribution onto the Hancock farm at the northern boundary. The specific sampling point was a faucet attached to the pipe connecting the pressure gauge in the top of the submerged distribution can. A 6-foot long, 3/8-inch diameter tygon tube was connected to the faucet and run outside Can 4's sheltering building to a 4-liter beaker at the back side. The composite sampler was set inside the building with its sampling tube running under the building's frame and into the beaker. At time of sampling, the faucet was turned on, and the wastewater flowed into the beaker and overflowed onto the adjoining field.

A new sampling location was added at the Hancock storage lagoons beginning in June 1982 after the reservoirs became operational. Since Reservoir 1 supplied most of the stored wastewater applied from reservoir during the summer 1982 irrigation season, samples were collected either as a composite of grabs from various depths and locations in Reservoir 1 or as a time-weighted composite from Can 1 at Reservoir 1 when irrigation from reservoir was occurring. During 1983 samples of the reservoir storage system consisted of volume-weighted composites based on historical and projected use from individual reservoirs or a 24-hour composite from Reservoir 1 when it was the only reservoir used for irrigation. An example is: if two reservoirs were contributing equally to irrigation with no irrigation direct from line, then the reservoir composite sample would be composed of water, 50% from each of the two reservoirs. If more than one reservoir was composited, then grab samples were obtained from the faucets at each reservoir's distribution can. If only one reservoir was being used for irrigation, then a 24-hour composite sampler was set up at the reservoir's distribution can similar to that for the pipeline sample.

The Wilson wastewater samples were obtained from the Wilson sewage treatment plant using an automatic sampler in a time-proportional operational mode. Initially, the effluent from the Imhoff tank (WIT) was sampled prior to the evaporation lagoons. On November 1, 1982 the Wilson sampling location was changed to the influent after the bar-screen and grit chamber and prior to the Imhoff tank inlet. This change was made to enhance recovery of viruses from this sample source. To collect a WIT sample, an ISCO Model 1580 automatic sampler was used in a time-weighted mode over a 24-hour collection since no flow measuring device was available. During collection the sampled wastewater was cooled to 4°C and at the conclusion of the 24-hour sampling period was transferred to sterile bottles and shipped with the LTFP effluent samples. A complete description of equipment used and sampling procedure is given in Appendix K.

Wastewater Aerosol

Background Runs—1980 Baseline Year--

Four background air sampling runs were performed in August 1980 before commencement of any spray irrigation at the Hancock farm. The objectives of these runs were twofold: 1) to estimate the air concentrations of the microorganisms of concern which residents in the area typically breathed when outdoors and 2) to identify whether there were any significant aerosol sources of these microorganisms besides the irrigation system planned for the Hancock site (e.g., the Wilson effluent pond). The first objective included determining background air concentration estimates both for Wilson and for the rural area. The information collected from these runs aided in the selection of microorganism groups to monitor on the other types of aerosol runs. Additionally, background exposure information was an important component of a balanced overall assessment of the significance of participant exposure to a given microorganism concentration due to wastewater aerosol sources.

These runs were conducted on four consecutive days during the period August 5 through 8, 1980. Aerosol samples were collected by operating nine Litton Model M large volume samplers (LVS) simultaneously for 30 minutes before sunrise (0630 to 0700) at nine locations in or near the Hancock farm. Locations for samplers included three within the city limits of Wilson, one downwind of the Wilson effluent pond, one at a farm household near the center of the Hancock farm, and the remaining four at farm households in quadrants of the study area. Specifically, the sampler locations as shown in Figure 8 were as follows:

> Wilson: Three samplers were placed in fixed predetermined locations (A, B, C) in the backyards of three Wilson families in the health watch. These samplers were 400 meters apart, with residences in all directions from each sampler location.

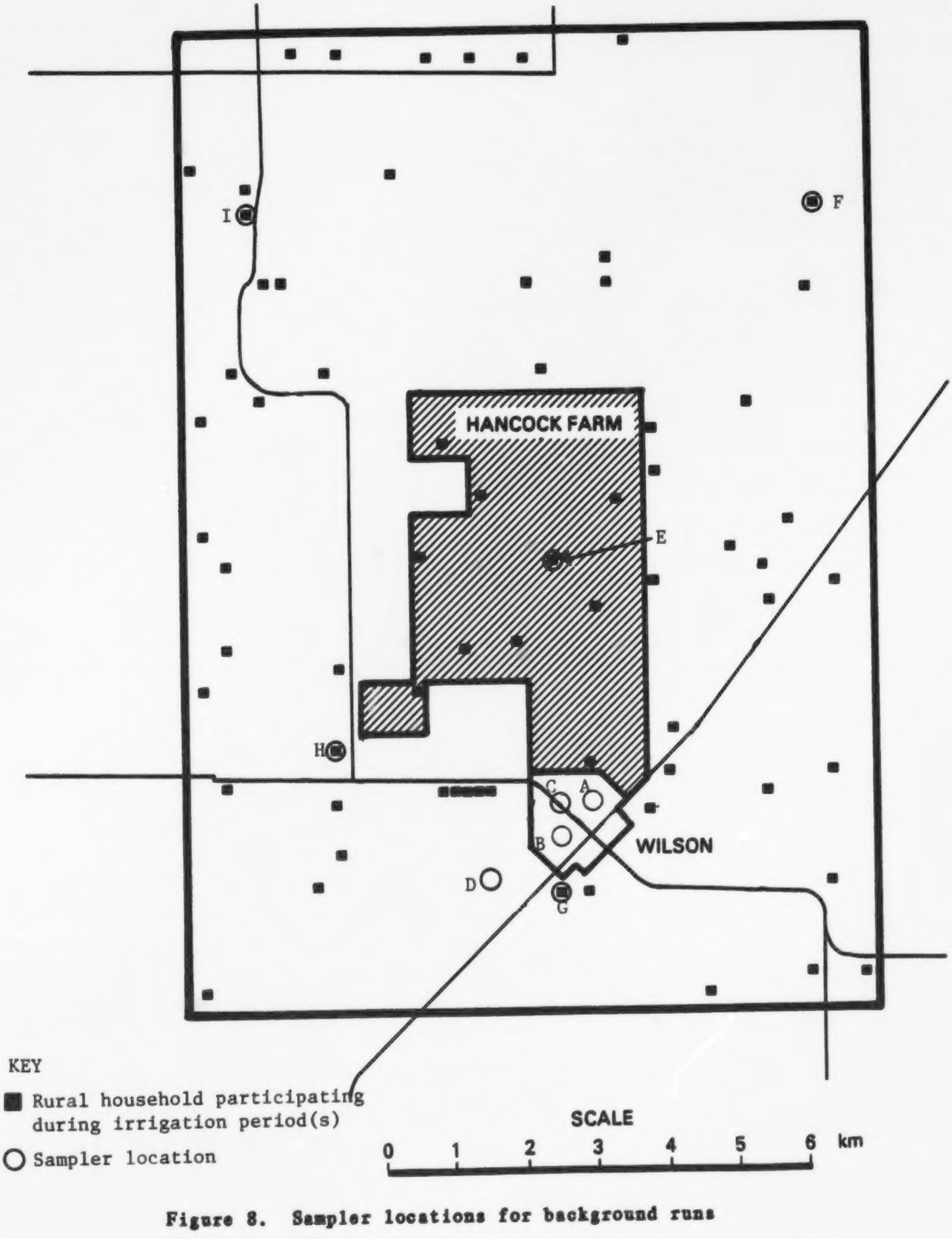

HANCOCK FARM
WILSON
A
B
C
D
E
F
G
H
I
KEY
■ Rural household participating during irrigation period(s)
○ Sampler location
SCALE
0
1
2
3
4
5
6
km

Figure 8. Sampler locations for background runs

Wilson effluent pond: One sampler was located downwind from the middle of the first effluent pond, 13 meters from the pond edge (Location D).

Rural area: Five samplers were placed in fixed predetermined locations approximately 10 meters upwind of the homes of five rural families participating in the health watch:

E - farm near center of Hancock site
F - farm in northeast quadrant (4 km NE of Hancock site)
G - farm 0.7 km south of Wilson (upwind)
H - farm in southwest quadrant (<1 km SW of Hancock site)
I - farm in northwest quadrant (3.5 km NW of Hancock site).

Each sampler location was in an open area at least 10 meters from any house, farm, or lane. No obvious sources of microorganism aerosols were located near or upwind of any selected locations near homes. Cotton was growing on all nearby farmland. There were no cattle or horses at or within a kilometer upwind of any sampler location. There were hogs near locations D and H, but they were never upwind during sampling. A few household and farmyard animals (dogs, cats, chickens, etc.) were observed at nearly all sampling locations. Sampler operators wore surgical masks and usually stayed downwind during the air sampling to minimize their effect.

A grab sample of wastewater was taken near the middle of the large, shallow Wilson effluent pond after each run. During the week of sampling, the effluent was being diverted to an adjacent pond along a ditch about 12 meters upwind of the air sampler locations. The fecal microorganism levels were much lower in the pond than in the Imhoff tank effluent.

The wind was from the south-southeast (160° to 168°) on all four background runs. Winds were fairly strong on Run 2 (5.8 m/sec), but light on the other runs. Solar radiation was nil (<15 W/m^2) since sunrise was at 0703. Temperature ranged from 19°C to 23°C, while the relative humidity varied from 69 to 76%.

Litton Model M large volume samplers were selected for performing both the background runs and microorganism runs, primarily because the large volumes of air which can be sampled provide sensitivity to detect low microorganism levels in the air. These samplers were designed to collect airborne particles by electrostatic attraction to a rotating disk on which they are concentrated into a thin, moving film of collection media. A complete description of the sampler is provided in Appendix L. Collection efficiencies for electrostatic precipitators depended on the operating high voltage. Sufficient voltage must be supplied to produce a particle charge; the greater the voltage, the greater the driving force (particle charge) to effect particle separation from air. However, very high voltages produced sparking which in turn disrupted the electrical equipment and electrodes, reducing the effective voltage.

Field operation of the samplers first required that an effective decontamination be performed followed by suitable storage in this sterile state.

This was accomplished by a cleanup procedure using both absolute ethanol and a buffered Clorox solution, followed by sealing all sampler openings. All decontamination procedures, both before commencement of any aerosol run attempts and at the conclusion of each aerosol run, were performed in a laboratory at LCCIWR. A copy of the step-by-step cleanup procedure is found in Appendix M.

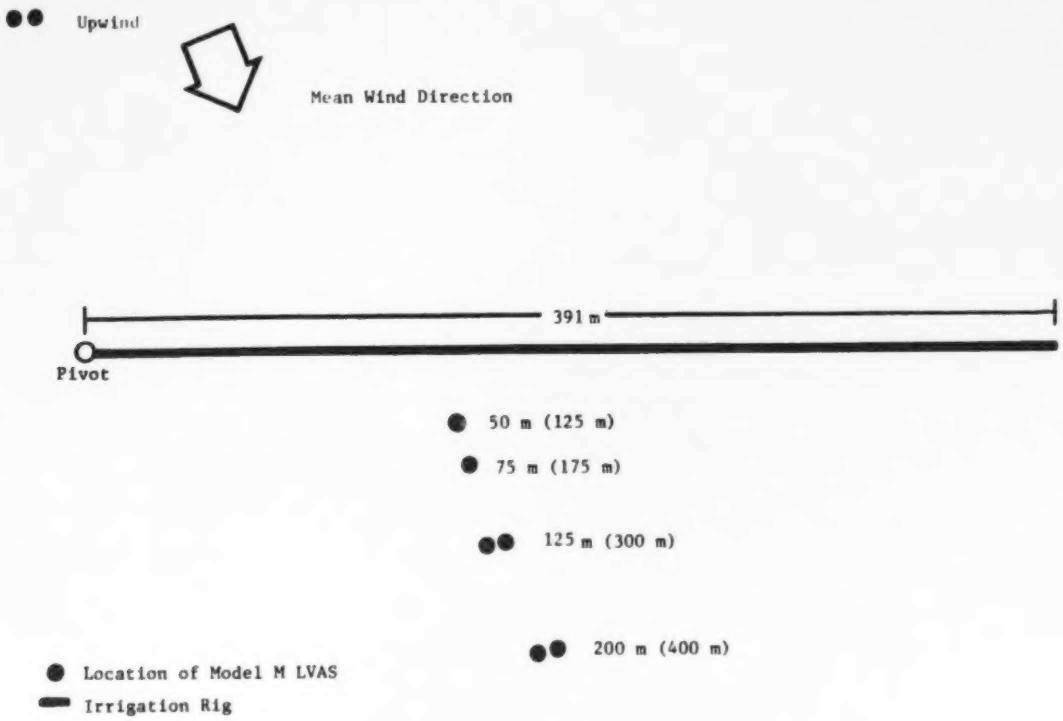
Sampler runs were initiated by placing the necessary equipment with an operator at each sampling site prior to the preagreed start time of 0630. At each site the operator placed the LVS on a table which was leveled by means of adjustable legs and connected an extension cord to a nearby power source. At all sampling sites except the Wilson effluent pond where a gasoline-powered alternator was used, arrangements were made to operate samplers from a local power outlet. By a predetermined arrangement and synchronization of watches, all operators started sampler operation at 0630. During these runs sampler operational parameters included an air flow rate of 1000 liters per minute (1.0 m^3/min), a high voltage setting of 12 to 15 kV (highest voltage obtainable without significant sparking) and a minimum recirculation rate of brain-heart infusion (BHI) broth, the collection media, of 10 mL/min. BHI with 0.1% Tween 80 to prevent foaming was selected as the collection and transfer medium. This medium has previously been shown to be adequate for sample concentration and for preservation and assay of the microorganisms (Johnson et al., 1980). At the conclusion of each sampling run, media containers were tightly capped, appropriately labeled, cooled to 4°C, and immediately shipped to San Antonio via commercial airline counter-to-counter parcel service. Sample analyses were initiated the same day as sample collection.

Wastewater Aerosol Monitoring--1982 Irrigation Year--

In 1982, aerosol monitoring of spray irrigation rigs was conducted during five monitoring periods covering 6 weeks of irrigation: 2 weeks during spring irrigation and 4 weeks during summer irrigation. Five types of aerosol runs comprised aerosol sampling: microorganism runs, quality assurance runs, virus runs, particle size runs, and dye runs. Diagrams of typical layouts for each of these runs showing sampler locations relative to the aerosol source are shown in Figures 9 through 12.

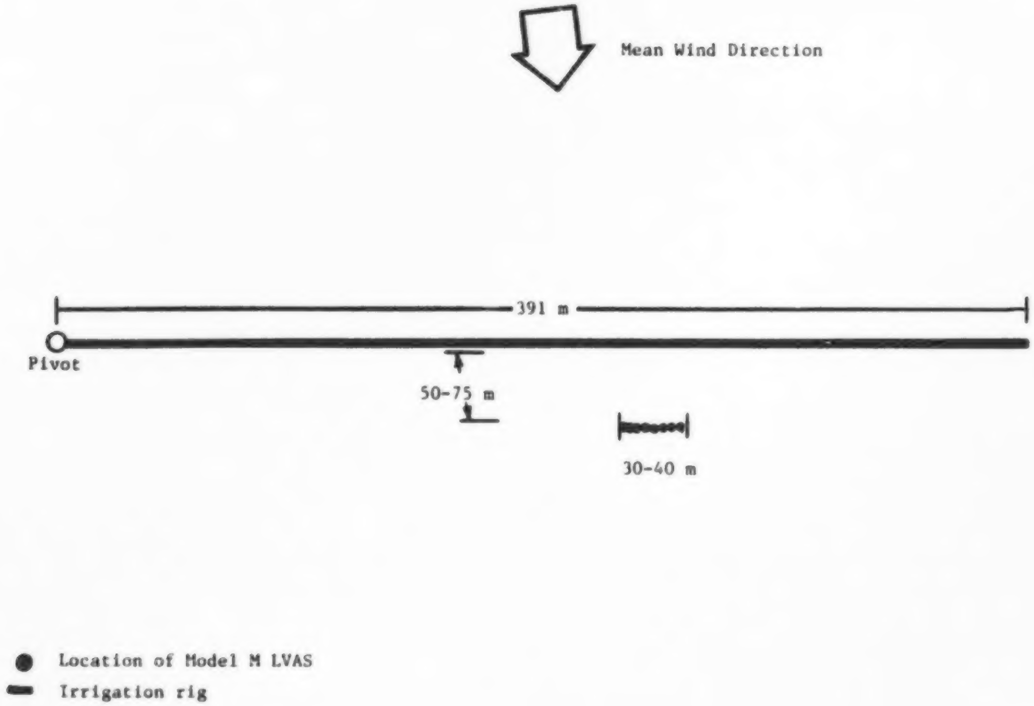
Microorganism runs--A total of 20 microorganism runs were completed during the preplanting and summer 1982 irrigation periods at the Hancock farm to characterize the wastewater aerosol. Results from these runs characterized microorganism densities in air under various conditions at the Hancock site at distances up to 400 meters downwind of the irrigation rig.

To conduct these runs, ten large volume aerosol samplers (Litton Model M) as used on the background runs were loaned by the Naval Biosciences Laboratory to SwRI under a subcontract. These were deployed at various downwind distances up to 400 meters from the rig sampled and upwind of the primary aerosol source sampled. Initially, samplers were located at nominal downwind distances of 50 m, 75 m, 150 m (paired), 200 m (paired) and at an upwind location (paired). Nominal downwind sampler distances were subsequently adjusted for some microorganism runs to 125 m, 175 m, 300 m (paired) and 400 m (paired) to determine microorganism aerosol levels



●● Upwind
Mean Wind Direction
391 m
Pivot
● 50 m (125 m)
● 75 m (175 m)
●● 125 m (300 m)
●● 200 m (400 m)
● Location of Model M LVAS
▬ Irrigation Rig

Figure 9. Typical sampler configuration for microorganism run



Mean Wind Direction
391 m
Pivot
50-75 m
30-40 m
● Location of Model M LVAS
▬ Irrigation rig

Figure 10. Typical sampler configuration for quality assurance and enterovirus runs

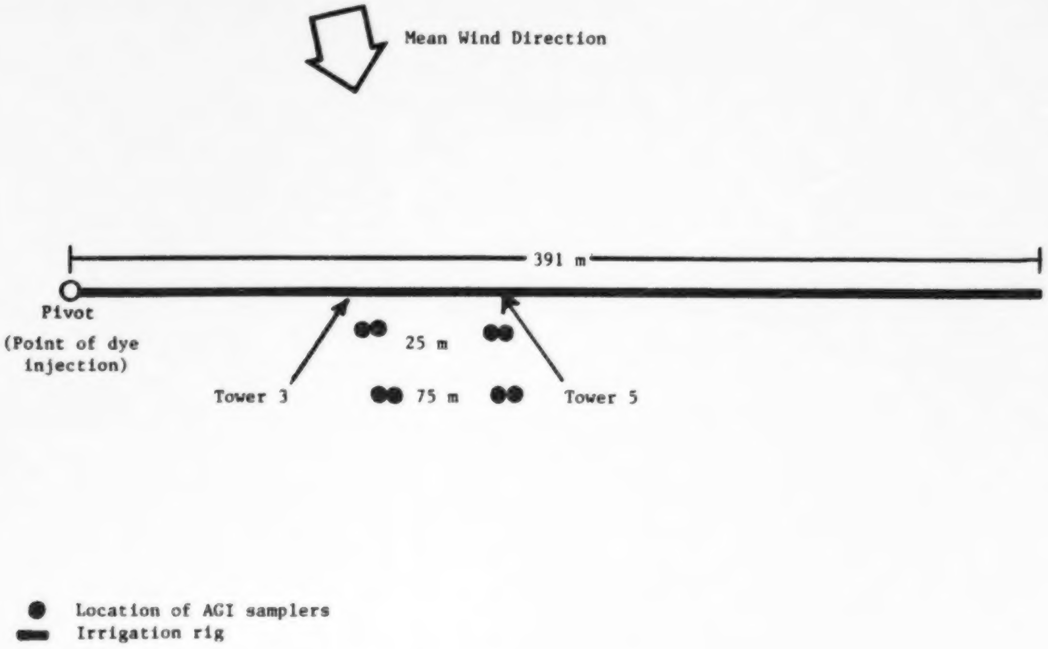

Mean Wind Direction
391 m
Pivot
(Point of dye injection)
25 m
Tower 3
75 m
Tower 5
● Location of AGI samplers
▬ Irrigation rig

Figure 11. Typical sampler configuration for dye run

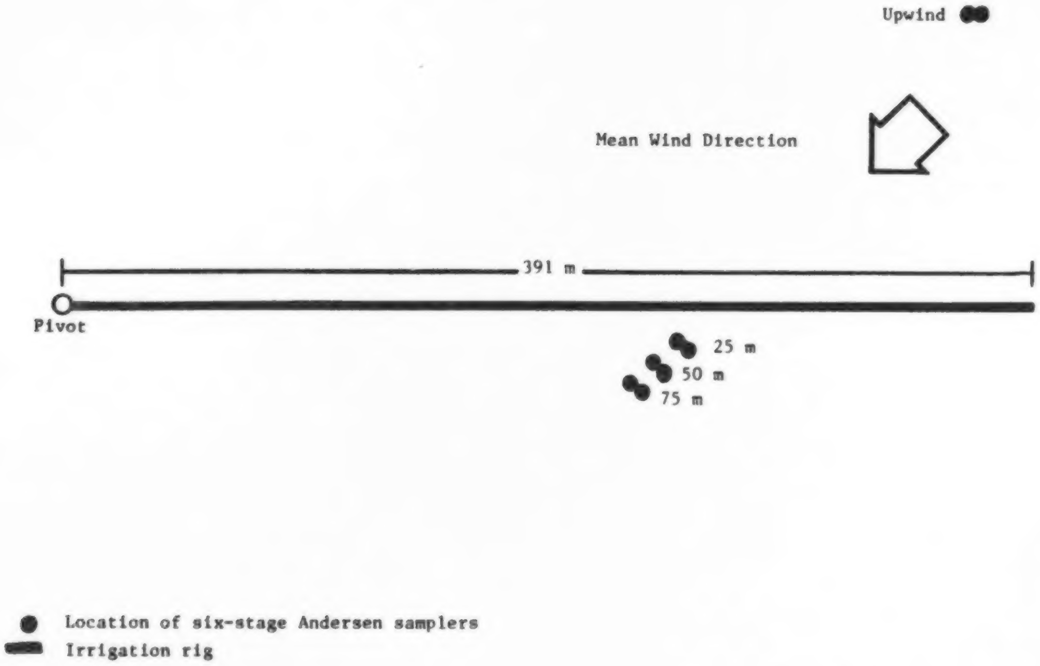

Upwind
Mean Wind Direction
391 m
Pivot
25 m
50 m
75 m
● Location of six-stage Andersen samplers
▬ Irrigation rig

Figure 12. Typical sampler configuration for particle size run

out to the 400-m buffer zone boundary. Actual sampling distances on each run are given in Table A-5 in Appendix A.

Model M samplers were decontaminated utilizing the same procedure used for the background runs. BHI plus 0.1% Tween 80 was again used as the sampling fluid. All runs consisted of a simultaneous 30-minute sampling time with sampler operation at 1.0 m^3/min air flow and maximum high voltage obtainable with minimal plate sparking. Field conditions occasionally required LVS operation below 12 kV to eliminate sparking. It was often difficult for field operators to maintain an average air intake flow rate for a run at 1.0 m^3/min, since sporadic wind gusts would temporarily alter the air flow rate.

During the time of aerosol sampling, a simultaneous wastewater composite sample was collected from the irrigation spray rig being monitored. At the completion of each run, samples were labeled, cooled to 4°C, and shipped to the UTSA-CART laboratories for analysis on the following day.

Sampling conditions for the microorganism runs are summarized in Table A-5 in Appendix A. The operating voltages of the large volume samplers during these runs are provided in Table A-6 in Appendix A.

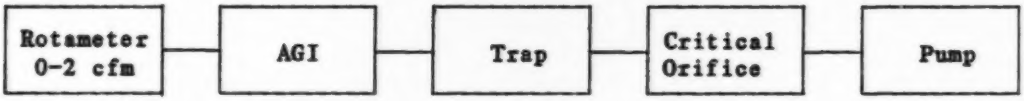
Quality assurance runs--Two quality assurance (QA) runs were conducted to determine assay variability between samplers, between aliquots of BHI from the same sampler and between replicates split by the receiving laboratory. These runs consisted of the same cleanup and operational protocols utilized for the microorganism runs with the exception that all operational samplers were lined up in a row (2-meter separation) equidistant and parallel to the orientation of the spray irrigation rig. For QA Run 1, conducted during spring irrigation at a time of blowing dust, the nozzle line to sampler line distance was 50 meters, whereas for QA Run 2, conducted during the summer irrigation period, the distance was 75 meters. Sampling conditions for the quality assurance runs are shown in Table A-7 in Appendix A. After aerosol collection, but prior to shipment to the CART laboratories, the 100-mL BHI aliquots from each sampler were split into four equal aliquots to achieve a blind distribution of ''identical'' samples for a predetermined sequence of microorganism assays.

Enterovirus runs--Since the wastewater contained a high enough level of enteroviruses for the microbiological dispersion model to predict their probable detection in aerosols, four special aerosol runs were conducted to estimate the enterovirus aerosol concentration. To conduct this type of run, all functional large volume samplers were operated simultaneously at a downwind distance of about 50 meters from the nozzle line for five consecutive 30-minute sampling segments. The samplers were aligned parallel to the nozzle line with a sampler spacing of 1.5 m. The irrigation rig was operated at a reduced travel setting so it progressed on the dry side of the field (i.e., toward the samplers) at some minimal rate, typically 5 to 10 m/hr at the tower used for alignment of the sampler line. At the end of every two 30-minute periods, the sampler position was adjusted to compensate for the rig movement. The initial and final distances from sampler line to nozzle line are shown for each segment on the sampling conditions summary presented in Table A-8 in Appendix A. The BHI collection fluid was changed after each sampling segment, and all BHI was pooled at the conclusion of the run. After transport to the UTSA-CART laboratory, the BHI was concentrated and plaque-assayed for human enteric viruses.

Dye runs—Four dye aerosol runs were conducted to estimate the aerosolization efficiency (i.e., the fraction of sprayed wastewater that becomes aerosolized) of the spray irrigation rigs at the Hancock site. The rig nozzles directed a fairly fine spray laterally and upward in a 360° umbrella pattern which appeared to enhance aerosol production and drift, due to improper design. Thus, it was anticipated that the Hancock farm aerosolization efficiency may differ substantially from the 0.33% (geometric mean) aerosolization efficiency of the impact sprayers used for wastewater irrigation at Pleasanton, California (Johnson et al., 1980).

One dye run was conducted in March 1982 while the last three were completed in July 1982. To perform these runs, a 20% solution of Rhodamine WT dye mixed with glycerol was injected at a constant rate into the pipeline supplying the sprayers of the irrigation rig being sampled. The dye was injected with a Zenith Constant Torque Unit Type ZM coupled with a No. 11 Zenith Metering Gear Pump.

Aerosols were collected using 500-mL graduated all-glass impinger (AGI) samplers connected to a vacuum pump as indicated in the following schematic:



The rotameter was used only for calibrating the system in the laboratory. With the critical orifice in line and a pump vacuum of at least 15 inches mercury, the nominal flow rate through AGI sampler was 1.0 CFM (cubic feet per minute).

To perform a dye run, AGI samplers containing 100-mL deionized water as collection media were set up in pairs at four locations: two pairs at 25 m and two pairs at 75 m downwind of the monitored rig. One sample set (i.e., 25-m and 75-m pairs) was aligned with a tower near the center of the irrigation rig while the other sampler set was aligned at the same orientation but displaced to the right or left of the center line set depending upon wind direction by two rig spans. When all equipment was in place, the Zenith gear pump began injection of dye into the irrigation system, and when the dye was visible in front of a sampler set, the AGI samplers commenced operation. Samplers were operated until dye was no longer visible at the nozzles directly in front of the sampling station which typically was 6 to 7 minutes. At the conclusion of the sampling period, the water media was transferred to glass bottles for storage until analysis. As soon as dye was visible in the wastewater at the nozzle closest to the injection pump, grab samples were obtained at 1-minute intervals for as long as dye was visible to determine source strength. Dye concentrations in both the aerosol samples and wastewater samples were determined using a Turner Spectrofluorometer Model 430. Sampling conditions for the dye runs are displayed in Table A-9 in Appendix A.

Particle size runs—Five particle size runs were performed using Andersen 1 CFM six-stage particle samplers to determine the concentration and particle size distribution of the wastewater aerosol microorganisms. The samplers were connected to the orifice system and vacuum pump that was utilized on the dye runs to maintain a nominal flow rate of 1 CFM through the sampler. Each run was made with eight samplers deployed in pairs, one upwind of the sampled source and the remaining three at nominal downwind distances of 25, 50 and 75 meters. Sampling times ranged from 8 to 10 minutes. A summary of sampling conditions during each of the particle size runs is shown in Table A-10 in Appendix A. A composite wastewater sample was collected simultaneously from the rig being sampled to determine source strength.

Standard plate count agar was used as the collection medium in these samplers. After sample collection, plates were incubated at the LCCIWR laboratories for 24 hours at 35 ± 0.5°C and counted for colonies.

Dust storm runs--Dust storms that could entrain many sprayed microorganisms from the spray fields as a particulate aerosol are common in the Lubbock area, especially in spring. These dust storms may be another wastewater-associated pathway of infection in addition to the wastewater aerosol. If dust storms occurred during aerosol monitoring periods, special dust storm sampling runs were planned. These runs would have been performed by utilizing AGI samplers with BHI collection medium operated for a brief period (about 15 minutes). Samplers would have been located both upwind and downwind of the spray fields on each dust storm run.

No localized dust storms occurred during any of the air sampling weeks in 1982. However, QA Run 1 took place during a time of blowing dust. On this run, the aerosol levels of fecal coliforms and fecal streptococci were higher than expected, based on the results from the microorganism runs at the same downwind distance involving similar wastewater concentrations. It is possible that the approximately threefold increase in aerosolized fecal coliforms and the nearly doubled level of aerosolized fecal streptococci were due to the blowing dust.

Calculation of Microorganism Density in Air

The microorganism density sampled in air was calculated from the assayed microorganism concentration in the sampler's collection fluid. For an individual LVS, the equation is

$$C = \frac{A \times V}{F \times R \times D}$$

where C – concentration of detectable microorganism units/m^3 of air (e.g., cfu/m^3)

A – concentration of detectable microorganism units assayed in the collection fluid (cfu/mL)

V – final volume of collection fluid (usually 100 mL)

F – correction factor for LVS operating voltage (reference basis of 12 kV)

R – air sampling rate (usually 1.0 m^3/min)

D – sampling duration (usually 30 min)

LVS were not as efficient as impinger samplers in the collection of microorganisms in air, and the efficiency varied with the LVS operating voltage. The collection efficiency of the LVS units employed in the field sampling was determined relative to AGI samplers in wind tunnel experiments performed in July 1980 and October 1982. The relative collection efficiencies (mean ± standard error) of the LVS were found in the 1982 tests to be 0.29 ± 0.017 in 18 tests at 12 kV and 0.68 ± 0.022 in 29 tests at 13 to 18 kV.

No attempt was made to adjust the aerosol concentration to the AGI collection efficiency since there is no standard aerosol sampling method and since the absolute collection efficiency of AGI samplers was not determined. Rather, the LVS data were corrected for operating voltage to render these data as internally consistent as possible.

The applied correction factors F for various operating voltages are presented in Table A-11 in Appendix A. These correction factors are the minimum expected correction. Appendix N presents the details on the calibration studies and evaluation of four candidate operating voltage correction factors. While other environmental factors such as particle concentration in air and relative humidity may also influence collection efficiency, no corrections have been applied to the aerosol data for such factors because the experimental data were insufficient to develop calibration curves.

The enterovirus density in air was determined during virus runs in which the collection fluid from many LVS was pooled and all except 100 mL of the fluid was concentrated prior to assay for enteroviruses. The enterovirus density in air equation is

$$C = \frac{B \times U}{\frac{(V - 100\ \mathrm{mL})}{V} \sum_{i=1}^{n} F_i \times R_i \times D_i}$$

where B – concentration of detectable enterovirus units in concentrated collection fluid, pfu/mL

U – final volume of concentrated collection fluid, mL

V – final volume of pooled collection fluid, mL

n – number of LVS samplings pooled.

For particle size aerosol runs the number of viable aerobic particles per unit volume of air for each stage in the sampler was calculated using the formula

$$C = \frac{A}{R_o \times T \times 0.0283}$$

where C - concentration in air, cfu/m^3

R_o - sampling rate for system from calibrated orifice, (range of 0.82 to 0.91 ft^3/min)

T - sampling time in minutes

0.0283 - conversion factor for ft^3 to m^3.

Results for each stage were reported as cfu/m^3 which represented the mean number of viable particles detected on standard plate count agar per cubic meter of air sampled.

The concentration of Rhodamine dye in the aerosol collected in each downwind impinger during the dye runs was calculated using the formula

$$C = \frac{C_I \times V}{R \times T \times 10^3}$$

where C - concentration in air (μg/m^3)

C_I - Rhodamine concentration in impinger (μg/mL)

V - volume of impinger solution (usually 100 mL)

R - air sampling rate in L^3/min

T - sampling time in minutes

10^3 - conversion factor for L^3 to m^3.

The geometric mean of all applicable aerosol density values was used to estimate the middle of the aerosol density distribution in summary tables. When all values were below the detection limit, the estimate reported in place of the geometric mean was less than the cumulative detection limit obtained by pooling the total volume of air sampled. Sometimes the set of aerosol data included some measured values and some values below the detection limit. In such cases 1) the geometric mean was calculated with x/2 substituted for <x to handle densities below the detection limit, 2) the arithmetic mean was calculated with zero substituted for <x for densities below the detection limit, and 3) the geometric mean of the geometric and arithmetic mean values was calculated and reported in the summary table.

Flies

The health watch and ensuing data analysis had the ability to detect an increased incidence rate of illness/infection if it occurred in association with irrigation periods. If the rate decreased with distance from the spray fields, a very important public health consideration would be knowledge of the pathway or pathways by which the agent was introduced. Other wastewater-associated pathways could produce a distance-related pattern very similar to that of the wastewater aerosol, thus causing the aerosol to be blamed. Other pathways include: 1) dust storms (discussed above); 2) vectors (e.g., flies attracted by the wastewater lagoons); 3) rodents (e.g., feed or food stuffs contaminated by fecal droppings or urine from field mice, infected by wastewater spray, which may be spending the winter in farmhouses and barns); and 4) fomites (e.g., wastewater-contaminated work clothes or door-knobs). Since the possibility of a fly-insect vector pathway of infection is frequently cited and the cost was low, a small pilot study was conducted to investigate this potential route of transmitting infectious agents. However, lacking an illness/infection distance pattern, the cost of investigating such other pathways of infection as rodents and fomites could not be justified.

Houseflies and other flies were trapped at the farmhouses and at effluent ponds. Using baited traps, flies were collected next to a pig pen near the Wilson sewage treatment facility and at the several farmhouses in 1980, collection attempts were made at the reservoirs and at farmhouses in 1982, and flies were collected in the irrigated fields, at Reservoir 1, and at the pig pen near the Wilson sewage treatment facility in 1983. An effort was made to isolate and quantitate the level of enteric bacteria and viruses in these fly samples. A target number of at least 200 flies per sample was sought (100 for bacterial analyses and 100 for viral analyses).

To collect flies, a stationary, bait-type trap was located and anchored in a potentially fly-prone area protected from wind, direct sunlight, children, animals and other potential disturbances. These traps were baited with a nonpoisonous bait such as canned cat food and milk. The cat food provided a perch for the fly to light on and the milk kept it moist longer since dried-up bait did not attract flies. The traps were checked every 24 hours at which time the bait was changed since fermented bait (with only milk added each day) may be harmful to farm pets.

When at least 200 flies were in the trap, it was placed in a large garbage bag and returned to the laboratory at LCCIWR. Initially (August 1980), flies were killed by using ether, but since this procedure was potentially detrimental to the bacteria of interest, it was discontinued. Thereafter the entire garbage bag and trap were chilled in a cold room (4°C) for at least 1 hour. The contents of each trap were emptied on paper, odd species of flies were discarded, and a maximum of 200 flies was counted out from each trap. The flies were transferred to a sterile container, appropriately labeled, and maintained at 4°C until arrival in the UTSA laboratory.

Drinking Water

Samples from drinking water sources on and surrounding the Hancock farm and the potable water for Wilson were collected and analyzed for total and fecal coliforms, fecal streptococci, and Salmonella. A total of 13 drinking water wells and one treated drinking water source was sampled periodically beginning in October 1981. Eight additional drinking water sample locations, including seven from households in the low exposure group, were added in December 1982 to provide representative data for the entire rural study area. The original sample locations plus those added are shown in Figure 13.

For most locations, samples were obtained from the cold water faucet on the kitchen sink of the residence. When samples could not be obtained from the kitchen faucet, an outside faucet was used. In either case, the faucet was cleaned with the tap water by hand using sterile polyethylene gloves. The outside of the faucet was scrubbed and the inside was cleaned within finger reach. Then the water was allowed to run for 5 to 10 minutes to flush loosened debris before collecting 1 liter of water in 1-liter, autoclave-sterilized, wide-mouth, screw-cap, polyethylene containers. After sample collection, the sample container was labeled with the sampling site and placed in an ice chest containing cold packs. Eight to ten samples were collected per day before the samples were transported to the LCCIWR lab for immediate sample analysis. Drinking vessels, refrigerated water, and other beverages were not tested.

Meteorological Data

Background Aerosol Runs--

Various meteorological parameters were observed and recorded during the four background runs conducted August 4 to 8, 1980 to quantify background air levels of microorganisms and to identify potential aerosol sources other than the spray irrigation system. These parameters included wind direction and wind speed at a 2-meter height utilizing a Meteorology Research, Incorporated (MRI) Model IM-5810 Mechanical Weather Station, temperature and relative humidity using a Bendix Psychron Model 566-2 psychrometer, and solar radiation using a Belfort Pyrheliograph 5-3850. All of these parameters were measured at the research plot near the center of the Hancock farm during the actual run time. Additional parameters obtained from the National Weather Service at Lubbock included time of sunrise, wind speed, wind direction, cloud cover, cloud type, and minimum height.

General Climatology--

An electronic weather station (EWS) and cassette data acquisition system (CDAS) from Climatronics Corporation were installed at the intensive research plot in March 1981 to measure and record general climatological parameters on the Hancock farm. Sensors to measure wind speed and wind direction were mounted on a 10-meter telescoping tower while sensors for measuring temperature, dew point, and solar radiation were located on a 2-meter tripod adjacent to the tower. These parameters were recorded continuously on a 5-inch wide chart moving at 1 inch per hour. Instantaneous values of these parameters were recorded every 5 minutes on a magnetic cassette tape in the CDAS unit. These tapes allowed cost-effective digitizing of meteorological data for the irrigation periods. For example, tables of hourly averages for all parameters plus wind rose plots were obtained.

The meteorological data accumulated in 1982 and 1983 on the CDAS was processed for the irrigation periods by Envirodata Corporation to produce hard-copy outputs of hourly averages, daily averages, and daily high and

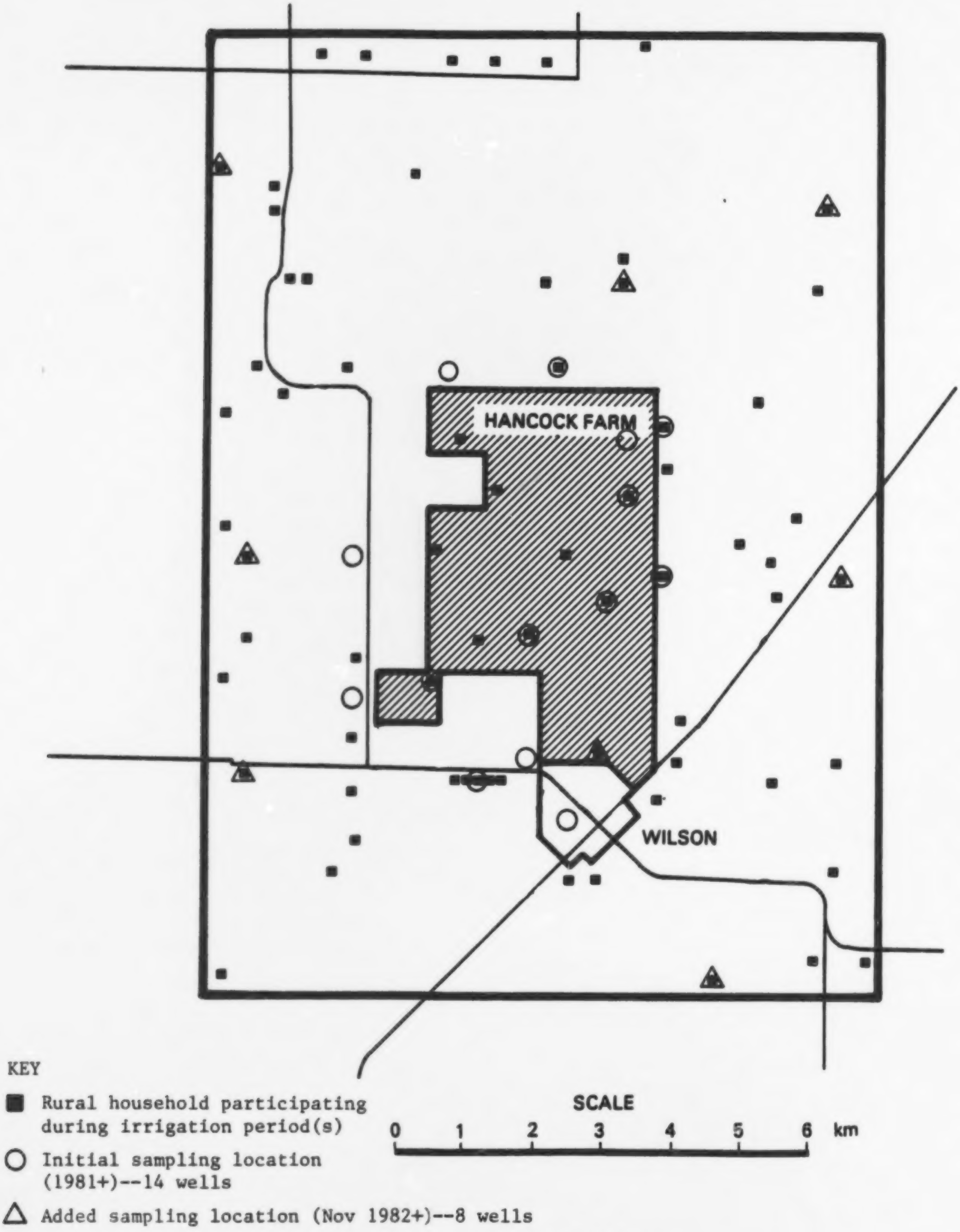

HANCOCK FARM
WILSON
KEY
■ Rural household participating during irrigation period(s)
○ Initial sampling location (1981+)--14 wells
△ Added sampling location (Nov 1982+)--8 wells
SCALE
0
1
2
3
4
5
6
km

Figure 13. Drinking water sampling locations

low values. Wind speed and wind direction data were processed for both 1982 and 1983. Solar radiation, temperature, and dew point data were processed for 1982 only. Wind rose plots for both the spring and summer irrigation periods for 1982 and 1983 were generated as shown in Figures A-3 to A-6 in Appendix A. No wind speed data for the 1982 summer period was plotted due to a malfunctioning anemometer translator board during most of this period.

Meteorological Measurements During Aerosol Runs--

During aerosol runs, meteorological parameters were measured about 100 meters downwind of the sampled rig to complement measurements made at the research plot by the Climatronics EWS/CDAS units. Field measurements included wind speed and wind direction at the 2-meter level, ambient temperature and relative humidity, and solar radiation by the same instrumentation utilized on the background runs. Visual observations were made for cloud type (to determine minimum cloud height) and eighths of the sky with cloud cover. The Climatronics CDAS unit was programmed to scan and record at 1-minute intervals during periods of aerosol sample collection.

Summaries of meteorological conditions for the different types of runs are presented in Tables A-12 through A-16 in Appendix A. Values for the EWS are averages obtained from the strip chart for the run period.

B. LABORATORY ANALYSIS OF SERUM AND CLINICAL SPECIMENS

Serology

Table 8 lists the epidemiologic charactersitics of the agents which were initially considered for use as serologic antigens in this study. Table 9 lists the antigens which were selected for testing; also listed, are the sera which were used for each of the selected antigens. With the exception of Influenza A and Legionella, all of the listed antigens are human viruses which infect the gastrointestinal tract, are excreted in the feces, and are known, or suspected, to be present in wastewater. None of the viruses selected were considered to be rare or geographically restricted.

Enteroviruses--

Initial sera from all study participants were tested for neutralizing antibody to the three poliovirus types. Individuals having low titers (<8) to any of the three polioviruses were recommended for immunization prior to the onset of irrigation. The remaining enteroviruses (Coxsackieviruses A9, B2, B3, B4, B5 and Echoviruses 1, 3, 5, 9, 11, 17, 19, 20, 24) were selected for use in the study according to the following criteria:

- The enterovirus was isolated from either Lubbock or Wilson wastewater (except Echoviruses 9 and 17).
- Stock virus for preparation of working virus suspensions was readily available from either ATCC (American Type Culture Collection) or CDC.
- The virus produced cytopathic effect (CPE) in Vero cells (except Coxsackievirus A9 which was grown in RD cells).

TABLE 8 EPIDEMIOLOGIC CHARACTERISTICS OF CANDIDATE AGENTS FOR SEROLOGIC TESTING

Virus and type	% Antibody prevalence[a]	Associated disease or symptoms	Isolation from stool	Occurrence in wastewater	Seasonal occurrence	Lubbock-Wilson wastewater
Hepatitis A	45	Inapparent, hepatitis		Yes	Fall/Winter	ND
Poliovirus 1	80	Inapparent, paralysis	Yes	Yes	All year	Yes
2	85	Inapparent, paralysis	Yes	Yes	All year	Yes
3	75	Inapparent, paralysis	Yes	Yes	All year	Yes
Coxsackie A1	Rare	Inapparent, orphan		Yes	Fall	Yes
A5	Rare	Rash, herpangina		Yes	Fall	No
A7	Rare	GI		Yes	Fall	Yes
A9	60	Rash, GI	Common	Yes	Fall	Yes
A10	Rare	Rash, pharyngitis		Yes	Fall	Yes
A16	25	Rash pneumonia	Yes, sporadic	Yes	Fall	Yes
B1	25	Pleurodynia	Seldom	Yes	Fall	Yes
B2	60	Colds, systemic	Common	Yes	Fall	Yes
B3	50	Colds, systemic	6-year epidemic	Yes	Fall	Yes
B4	70	Colds, rash, systemic	Common	Yes	Fall	Yes
B5	20	Colds, rash, systemic	Common	Yes	Spring/Summer/Fall	Yes
B6	Sporadic	Meningitis				No
Echo 1	15	Inapparent	Rare	Common		Yes
3	25	Meningitis	Rare, epidemic	Common	Fall	Yes
5	10	Meningitis	Sporadic	Yes	Summer	Yes
6	40	GI, meningitis	Common, epidemic	Common	All year	Yes
7	50	Meningitis	Common	Yes	Fall	Yes
9	55	GI, pneumonia	Most common, no epidemic	No		No
11	15	GI, cold	Common, no epidemic	Common	Winter	Yes
12	35	GI, rash	Rare, epidemic			Yes
13	15	GI	Rare, epidemic			Yes
14	15	Encephalitis	5-year cycles	Yes		Yes
15	15	GI		Yes		Yes
17	10	Meningitis	Sporadic	Yes		Yes
19	15	GI, pneumonia	Frequent	Frequent	Spring/Summer/Fall	Yes
20	15	GI, pneumonia		Yes		Yes
21	15	GI		Yes		Yes
24	15	GI, meningitis	Frequent	Yes		Yes
25	15	ARD	Frequent	Frequent	Fall/Winter	Yes
27	5	Meningitis	Rare	Yes		Yes
29	15	Meningitis	Frequent	Yes		Yes
30	15	Meningitis	Rare, epidemic	Yes		Yes
31	5	Meningitis	Rare	Yes		Yes
33	15	GI		Frequent		Yes
Adenovirus 1	40	GI	Yes	ND	Summer	ND
2	50	GI	Yes	ND	Summer	ND
3	50	Pharyngitis	Yes	ND	Summer	ND
4	20	ARD	Yes	ND	All year	ND
5	30	Pharyngitis, GI	Yes	ND	Summer	ND
6	10	GI	Yes	ND	Summer	ND
7	10	ARD	Yes	ND	Winter	ND
12	75 (adults)	Inapparent	Yes	ND	All year	ND
14	20	ARD	Yes	ND		ND
Reovirus 1	50	Orphan	Yes	Yes	Winter	ND
2	50	Orphan	Yes	Yes	Winter	ND
3	50	Orphan	Yes	Yes	Winter	ND
Rotavirus 1-4	50	GI	Yes	Yes	Winter	ND
Norwalk 1	50	GI	Yes	Yes	Summer	ND
Legionella 1	10	Respiratory			Summer	No
Influenza A	70	Respiratory	No	No	Winter	ND

ND - not detectable by standard concentration/assay techniques ARD - acute respiratory disease GI - gastrointestinal illness

a References: Fox and Hall (1980); Szmuness et al (1977); Jackson and Muldoon (1973a,b,c); Blacklow et al (1976, 1979); Helms et al. (1980).

TABLE 9. AGENTS AND SERA SELECTED FOR USE IN SEROLOGIC TESTING

	Serum Collection Period							
Agent	Jun[a] 1980	Dec 1980	Jun 1981	Jan 1982	Jun 1982	Dec 1982	Jun 1983	Oct 1983
Adenovirus 3	X			X		X		X
Adenovirus 5	X			X		X		X
Adenovirus 7	X			X	X			X
Coxsackievirus A9	X			X				
Coxsackievirus B2	X			X		X		
Coxsackievirus B3	X			X				
Coxsackievirus B4	X			X		X		
Coxsackievirus B5	X			X		X		X
Echovirus 1	X			X	X			X
Echovirus 3	X			X		X		X
Echovirus 5	X			X	X			X
Echovirus 9	X			X	X			X
Echovirus 11	X			X	X	X		X
Echovirus 17	X			X		X		X
Echovirus 20	X			X		X		X
Echovirus 24	X			X		X		X
E. histolytica	X			X			X	
Hepatitis A	X	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]	X[b]
Influenza A	X		X		X		X	
Legionella 1			X		X		X	
Norwalk 1	X[c]			X[c]			X	
Poliovirus 1	X			X	X			
Poliovirus 2	X			X	X			
Poliovirus 3	X			X	X			
Reovirus 1	X			X	X	X		X
Reovirus 2	X			X	X	X		X
Rotavirus	X	X	X	X	X	X	X	X

[a] In cases where this blood was not available, the first blood obtained from the participant was used.

[b] These bloods were tested only if previous blood was found to be negative for antibody.

[c] These bloods were tested only if June 1983 blood was found to be positive for antibody.

It was determined in advance that testing for antibody to a specific coxsackie- or echovirus would be continued to the end of the study only when it was determined that less than half of the population had antibody to that virus. This was done in order to maximize the number of ''susceptibles'' and therefore to increase the chances of detecting a statistically significant number of infections in the population. Therefore, only partial results are available for Coxsackieviruses A9 B2, B3, and B4. Additional enteroviruses were added to replace the agents which were dropped.

The serum neutralization test was used to determine antibody titers for the enteroviruses. This test was selected because it is considered to be the most sensitive and specific serologic procedure for detecting antibodies to these particular viruses. In this study, sera were initially diluted (1:4 for poliovirus titers, 1:10 for coxsackie- and echoviruses), then serially diluted (1:2) in microtiter plates. A challenge dose (30-300 $TCID_{50}$) of virus and a suspension of Vero cells were added to each of the serum dilutions. The antibody titer was determined to be the highest initial dilution which inhibited the CPE of the virus.

Adenoviruses--

Since the bentonite adsorption technique which was used in this study did not allow adenoviruses to be isolated from the wastewater, three adenoviruses (Adenoviruses 3, 5, 7) were arbitrarily selected from Table 8 for use in this study. The serum neutralization procedure which was described for the enteroviruses was also used to detect antibody to adenoviruses. Hep-2 cells were used in this procedure.

Hepatitis A—

During the course of this study, no routine method was readily available to detect hepatitis A in wastewater. However, the presence of hepatitis A in wastewater was presumed, since it is known to be present in urine and feces during infection. Screening for hepatitis A antibody was performed on initial sera from all participants. Only sera from participants who were found to have no antibody were tested in subsequent blood collection periods. The analysis of sera for the presence of hepatitis A virus (HAV) antibody was performed with a commercially available RIA system marketed by Abbott Laboratories under the name of HAVAB. This test is based on the principle of competitive binding of anti-HAV in serum with radioactively tagged anti-HAV to HAV coated on a solid phase bead.

Influenza--

Influenza virus was included in this study as an epidemiologic control since it is not excreted in the feces and therefore would not be found in wastewater. Complement fixation was the test of choice for measuring influenza A antibody. Guinea pig complement and sensitized sheep erythrocytes were used in this test. The antigen for this test was obtained from CDC.

Legionella bacilli--

Legionella organisms occur in the environment and can cause epidemic and sporadic cases of Legionellosis in man. Of particular interest in this study was the fact that algae were present in storage reservoirs on the Hancock farm. Since it is known that Legionella organisms utilize algae as a natural medium (Tison, et al., 1980), it was assumed that the organisms could be present in aerosols when the stored wastewater was applied to the land.

The indirect fluorescent antibody (IFA) test was used to determine the presence of antibody to L. pneumophila serogroup 1. The IFA test is a ''sandwich'' immunofluorescence technique which uses a two-stage reaction procedure. In the first stage, the Legionella antigen of interest is overlaid with dilutions of animal antiserum or human serum; the slides are then incubated, washed and dried. In the second stage, fluorescent dye-labeled antibody (to the IgG contained in the animal or human serum which was applied in the first stage) is placed on the slide. In this manner, Legionella antigens are rendered fluorescent by positive sera which themselves are not labeled.

Norwalk virus--

Sera from 25 children (under the age of 10) and 11 high exposure adults (with a history of self-reported diarrhea during 1982) were tested for antibody to Norwalk virus. This serology was performed at Dr. Neil Blacklow's laboratory at the University of Massachusetts. The RIA test developed by Dr. Blacklow was used to detect the presence of antibody to Norwalk virus.

Entamoeba histolytica--

Based on a report by Doby et al. (1980) of a higher carriage rate of E. histolytica in sewer workers in France, paired sera from 189 participants were tested for antibody to E. histolytica. The testing was performed under the supervision of Dr. George Healy, at CDC. Indirect hemagglutination was used to detect the presence of antibody.

Reoviruses--

Since reoviruses are commonly isolated from wastewater, all three human types were recommended for serologic testing. The hemagglutination-inhibition (HI) test was used to determine reovirus antibody levels. Antigen for this procedure was provided by the Biological Products Division of CDC.

In order to remove nonspecific inhibitors of hemagglutination, sera were pretreated with kaolin extract. Unfortunately, this treatmment caused the reovirus 3 agglutination pattern to ''collapse'' prematurely each time the test was run and titrition endpoints were unreliable. Therefore, only serology results for reoviruses 1 and 2 were used in this study.

Rotavirus--

Paired sera from 44 study participants under the age of 18 and 10 adults from the high exposure area (with a history of diarrhea in 1982) were tested for antibody to rotavirus. These reo-like viruses are known to cause sporadic and epidemic outbreaks of enteritis in children.

Rotavirus antibodies were measured by the enzyme-linked immunosorbent assay (ELISA). The supply of WA rotavirus stock antigen obtained from Dr. G. William Gary, CDC, Atlanta, was prepared in MA-104 cells. An ELISA plate reader was used to measure the spectrophotometric reaction.

Clinical Bacteriology

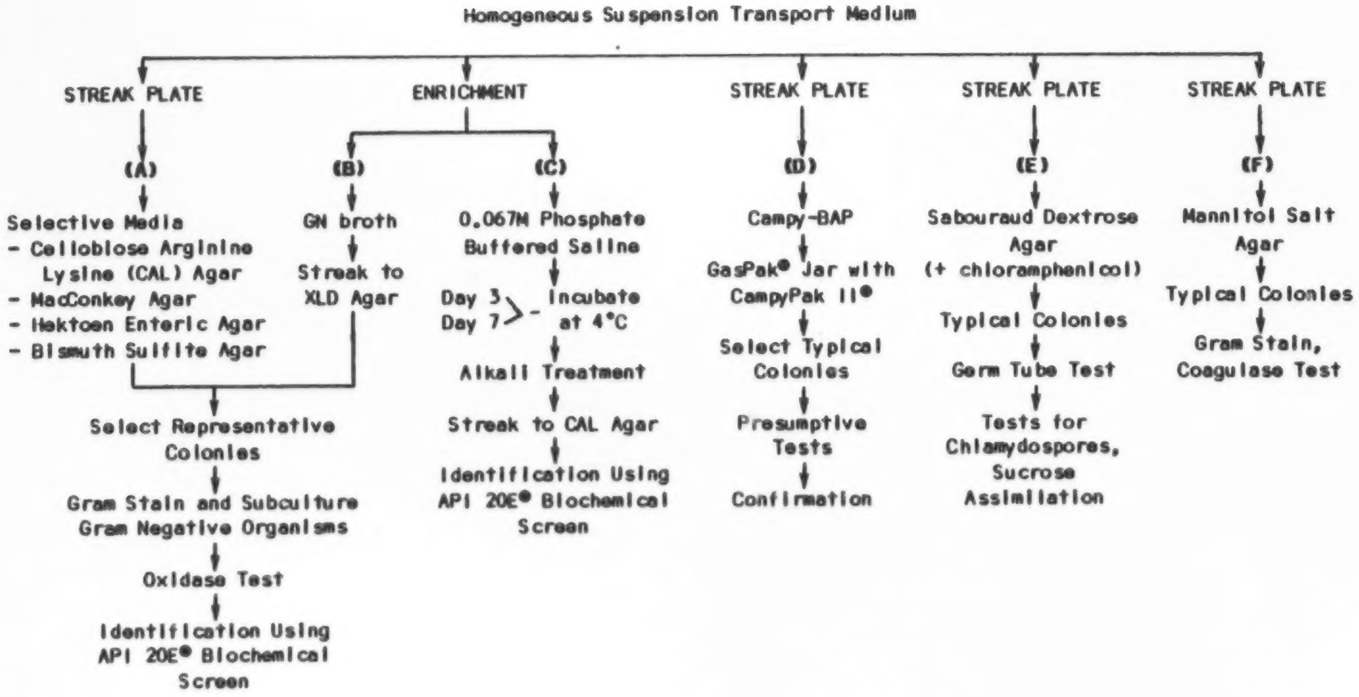
Analyses for selected organisms in fecal specimens and throat swabs were performed as summarized in Figures 14 and 15. The prevalence of different microbial types in the specimens was determined in a semiquantitative manner. All primary plating media were streaked by the same four quadrant method, and the amount of growth of each microorganism isolated was reported by determining the highest quadrant in which the organism was isolated as discrete colonies. The terminology used and respective definitions were:

Very light (VL) - 1 to 10 colonies on the plate
Light (L) - growth in first quadrant
Moderate (M) - growth on first two quadrants
Heavy (H) - growth on three or all quadrants

Fecal specimens which failed to yield any growth, or which yielded organisms by enrichment only, were excluded from the data set. The lack of organisms, in these cases, is likely to have been due to problems with sample processing, shipping or use of antibiotics by participants.

Fecal specimens in the transport medium were used for all isolations, with the exception of that for *Campylobacter jejuni* (Figure 14) where the specimen cup containing the representative portion of the original sample was used. Contrary to some reports (Lennette et al., 1980), *C. jejuni* may survive poorly in buffered glycerol saline (Sack et al., 1980), a widely used transport medium for most enteric bacterial pathogens. All media were formulated from the appropriate Difco (Detroit, Michigan) dehydrated product, with the exceptions of the cellobiose arginine lysine (CAL) agar of Dudley and Shotts (1979) which was obtained from Scott Laboratories (Fiskeville, Rhode Island) and plates of Campy-BAP agar which was purchased from the same source (Aldrich Scientific, San Antonio, Texas) or from BBL Microbiology Systems (Cockeysville, Maryland).

The procedure for primary isolation and identification of *Salmonella*, *Shigella*, *Yersinia enterocolitica*, and other enteric bacteria is shown in Figure 14. CAL agar, a special purpose differential medium for isolation of *Y. enterocolitica*, was incubated at room temperature for 48 hours. The other three media were chosen to represent three levels of selectivity for the various *Enterobacteriaceae* and other enteric organisms. These were a differential medium with little selectivity (MacConkey), a differential, moderately selective medium (Hektoen enteric), and a highly selective medium (bismuth sulfite) used primarily in the search for *Salmonella* (Lennette et al., 1980). All plates were incubated at 35°C and examined at 24 and 48 hours. Plates were inspected, using a stereomicroscope with oblique transmitted illumination, and a representative of every colony type observed was subcultured for a purity check, oxidase testing by Kovac's method (Lennette et al., 1980), and identification by the API-20E biochemical screen (Analytab Products). To increase the chance of isolating *Shigella* (Figure 14), 1 mL of each fecal specimen was transferred to 9 mL of GN broth, incubated at 35°C for approximately 18 hours, streaked to xylose-lysine-deoxycholate (XLD) agar, incubated at 35°C for 24 hours, and identified as described previously. The combination of enrichment in GN with isolation on XLD


Homogeneous Suspension Transport Medium
STREAK PLATE
ENRICHMENT
STREAK PLATE
STREAK PLATE
STREAK PLATE
(A)
(B)
(C)
(D)
(E)
(F)
Selective Media
- Cellobiose Arginine Lysine (CAL) Agar
- MacConkey Agar
- Hektoen Enteric Agar
- Bismuth Sulfite Agar
GN broth
Streak to XLD Agar
0.067M Phosphate Buffered Saline
Day 3, Day 7 - Incubate at 4°C
Alkali Treatment
Streak to CAL Agar
Identification Using API 20E® Biochemical Screen
Campy-BAP
GasPak® Jar with CampyPak II®
Select Typical Colonies
Presumptive Tests
Confirmation
Sabouraud Dextrose Agar (+ chloramphenicol)
Typical Colonies
Germ Tube Test
Tests for Chlamydospores, Sucrose Assimilation
Mannitol Salt Agar
Typical Colonies
Gram Stain, Coagulase Test
Select Representative Colonies
Gram Stain and Subculture Gram Negative Organisms
Oxidase Test
Identification Using API 20E® Biochemical Screen

(A) Salmonella, Shigella, Yersinia enterocolitica, other enterics
(B) enrichment for Shigella
(C) enrichment for Y. enterocolitica
(D) Campylobacter jejuni
(E) Candida albicans
(F) Staphylococcus aureus

Figure 14. Isolation and identification of selected organisms from feces

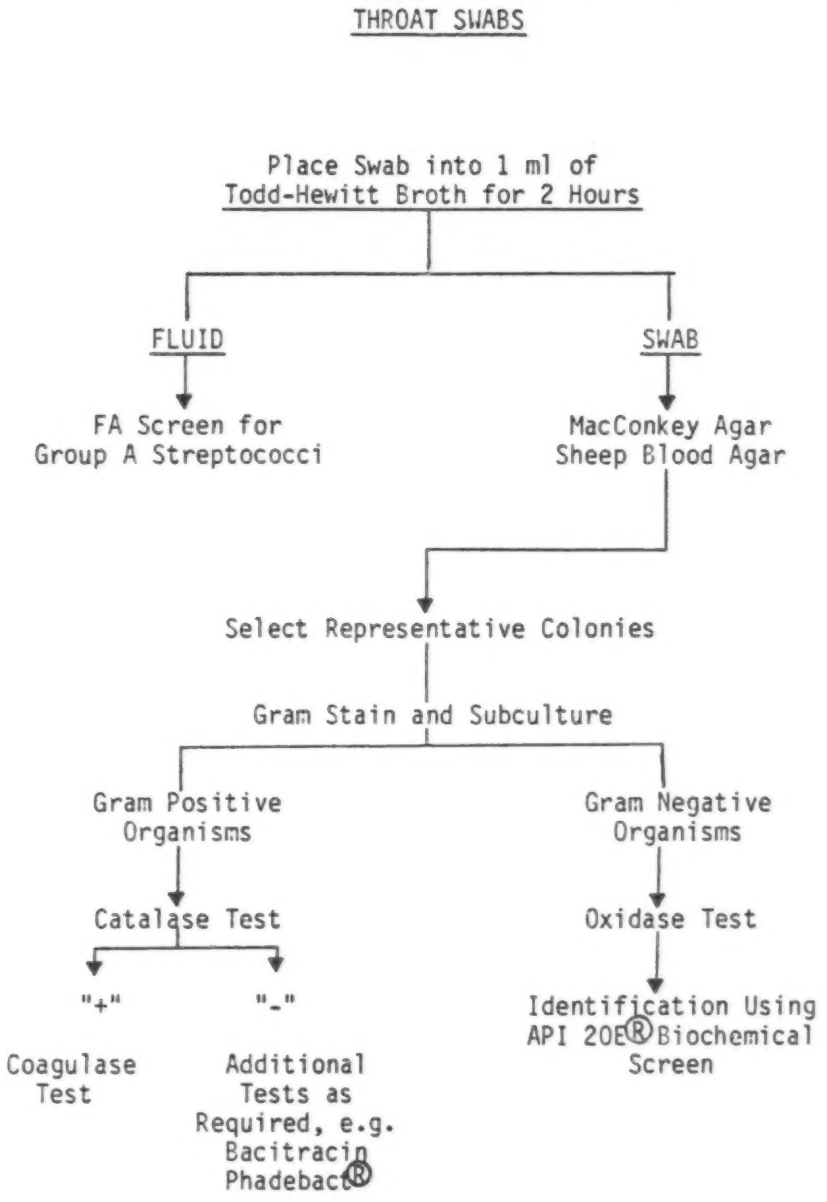

THROAT SWABS
Place Swab into 1 ml of Todd-Hewitt Broth for 2 Hours
FLUID
SWAB
FA Screen for Group A Streptococci
MacConkey Agar Sheep Blood Agar
Select Representative Colonies
Gram Stain and Subculture
Gram Positive Organisms
Gram Negative Organisms
Catalase Test
Oxidase Test
"+"
"-"
Coagulase Test
Additional Tests as Required, e.g. Bacitracin Phadebact®
Identification Using API 20E® Biochemical Screen

Figure 15. Isolation and identification of organisms from throat swabs

has been described as excellent for recovery of Shigella (Taylor and Schelhart, 1975).

Enrichment of Y. enterocolitica (Figure 14) was carried out by inoculating 1 mL of the fecal specimen to 9 mL of phosphate buffered saline followed by incubation at 4°C for 1 week. At Days 3 and 7, 10 µL of the enrichment was mixed in 0.1 mL of 0.5% KOH in 0.5% NaCl, and then streaked to CAL plates. Representative colonies were picked and identified, as described previously, after incubation at room temperature for 48 hours.

The procedure for C. jejuni involved streaking Campy-BAP plates, which then were incubated at 42°C for 48 hours in a GasPak container with the CampyPak II (BBL Microbiology Systems, Cockeysville, Maryland) atmosphere generator. The organism was presumptively identified by the following criteria: Gram-negative curved rods, characteristic darting motility, oxidase +, and catalase +. The organisms were confirmed by growth in 1% glycine, lack of growth at 25°C, and susceptibility to nalidixic acid (30 µg disk).

The fungal yeast pathogen Candida albicans was isolated (Figure 14) by streaking plates of Sabouraud dextrose agar supplemented with 50 µg/mL of chloramphenicol (Calbiochem), followed by incubation at 35°C for 48 hours. Plates were inspected for white, convex, opaque colonies which were confirmed as C. albicans by germ-tube formation in bovine serum, chlamydospore production on cornmeal Tween 80 agar, and sucrose assimilation on agar slants of Wickerham's yeast nitrogen base supplemented with the sugar.

Staphylococcus aureus was isolated by streaking on plates of mannitol salt agar. Mannitol-positive colonies were picked for confirmation by examination of Gram-stained smears for characteristic morphological groups of Gram-positive cocci and positive coagulase reaction.

Screening for C. albicans in stool specimens was initiated in September 1980 while the C. jejuni protocol was added in April 1981. The alkali treatment coupled with plating on CAL agar was substituted for an existing procedure in April 1981 for the improved detection of Yersinia enterocolitica. Prior to that time, fecal samples were analyzed for Y. enterocolitica by enrichment at 4°C in isotonic saline containing 25 µg/mL of potassium tellurite with subsequent plating onto Salmonella-Shigella (SS) agar.

Throat swab specimens (Figure 15) were plated onto 5% sheep blood agar and MacConkey agar. Incubation of the first medium was at 35°C for 24 hours in an atmosphere of 5% CO_2 to facilitate cultivation of Group A streptococci. The MacConkey agar plates were incubated at 35°C for 24 hours in normal atmosphere. Representative colonies from each medium were identified using traditional tests as described in Lennette et al. (1980) in conjunction with commercially available testing systems. Gram-negative organisms from MacConkey agar plates were identified using the API-20E (Analytab) system. Beta-hemolytic streptococci were grouped using the Phadebact (Pharmacia) coagglutination test. Throat swab specimens also were screened for Group A streptococci using a fluorescent antibody technique.

Clinical bacteriology monitoring, particularly of illness specimens, provided the most timely mechanism of surveillance for a possible health effect associated with irrigation operations. Isolation of a pathogen or any other cause for concern during periods of scheduled sampling was reported by telephone to health watch investigators at the University of Illinois within a week of receipt of the sample. The results of all illness specimens were reported by telephone within a week of receipt of the specimen. In addition, an illness specimen log, starting with specimens collected during DCP 212, was updated on the last Friday of each period and sent to the University of Illinois and the project manager. This mechanism of surveillance reporting allowed feedback of results to the participants and collection of follow-up specimens as appropriate.

Clinical Virology

Appropriate enteric and respiratory viral agents were sought via traditional diagnostic isolation schemes (as illustrated in Figure 16) coupled with microidentification techniques. Fecal suspensions were prepared by adding 10 mL of antibiotic diluent (Medium 199 containing penicillin and streptomycin) to 1 to 2 g of stool sample. Sterile glass beads were added, and the mixture was vortex-mixed for 1 minute. After centrifugation (8,000 x g) for 10 minutes in a refrigerated centrifuge, the supernatant fluid was recovered for inoculation of primate cells in tube culture. Similarly, an antibiotic diluent was added to the fluid expressed from the throat swab into the transport medium. If necessary, throat swab eluates were centrifuged to remove gross particulates prior to inoculation of cultures.

Cell cultures used were primary rhesus monkey kidney (Flow Laboratories, McLean, Virginia), human rhabdomyosarcoma (RD), African green monkey kidney (BGM) and HeLa (pretested for adenovirus sensitivity). A 0.1-mL aliquot of supernatant or eluate was inoculated into two tubes of each cell line. Tubes were observed microscopically over a 10- to 14-day period for viral CPE. HeLa cell tube cultures were frozen and thawed prior to a second blind passage to enhance detection of adenoviruses.

As a result of quality assurance testing conducted during 1981, it became obvious that the likelihood of recovering viruses from nonillness (routine) fecal specimens was low. Beginning with Period 201 sampling, changes in the clinical assay procedures were made to enhance the sensitivity of viral isolations from routine fecal specimens. The volume of sample inoculated into each cell line was increased from 0.2 mL to 1.0 mL by inoculating two 60-mm plates when monolayers reached 50 to 75% confluence (0.5 mL/plate). Primary rhesus monkey kidney cells obtained from a commercial supplier continued to be used as tube cultures.

The identification and typing of viral isolates from clinical specimens was performed by microneutralization using the Lim Benyesh-Melnick enterovirus typing pools (NIAID, 1972; NIAID, 1975). Fluorescein conjugated antisera specific for adenovirus group antigen was purchased from M.A. Bioproducts (Walkersville, Maryland). Preliminary testing showed that optimal fluorescence was obtained by using a 1:5 dilution of the conjugate. Prior to use, the

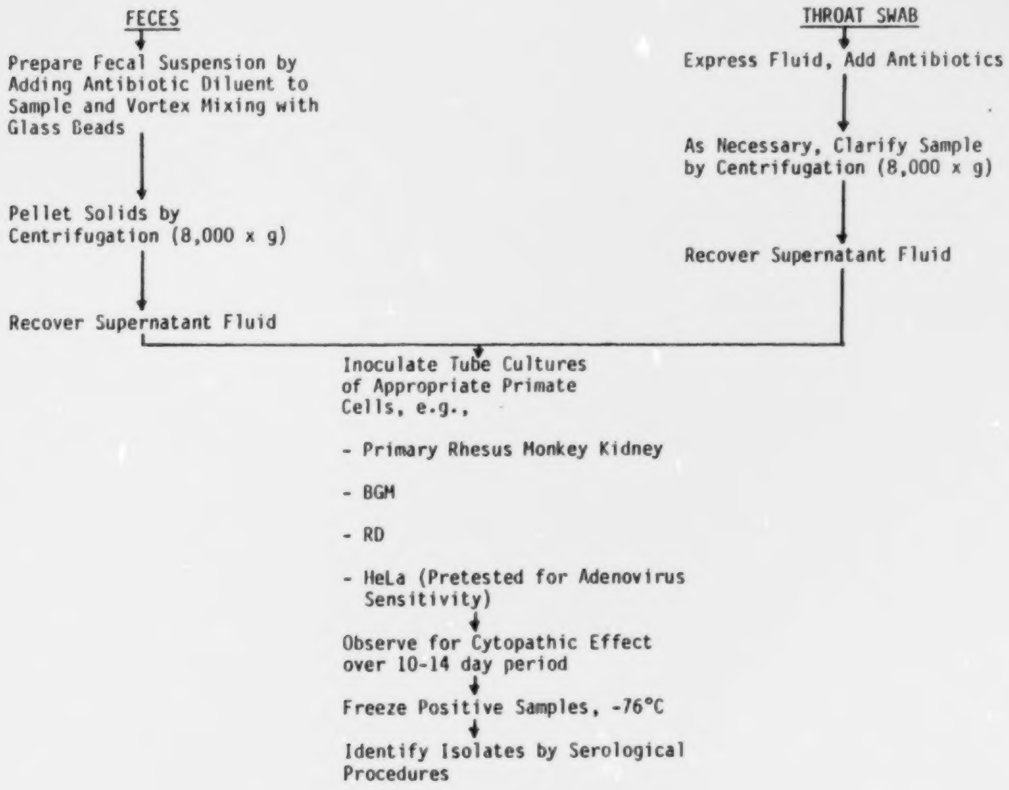

FECES
Prepare Fecal Suspension by Adding Antibiotic Diluent to Sample and Vortex Mixing with Glass Beads
Pellet Solids by Centrifugation (8,000 x g)
Recover Supernatant Fluid
THROAT SWAB
Express Fluid, Add Antibiotics
As Necessary, Clarify Sample by Centrifugation (8,000 x g)
Recover Supernatant Fluid
Inoculate Tube Cultures of Appropriate Primate Cells, e.g.,
- Primary Rhesus Monkey Kidney
- BGM
- RD
- HeLa (Pretested for Adenovirus Sensitivity)
Observe for Cytopathic Effect over 10-14 day period
Freeze Positive Samples, -76°C
Identify Isolates by Serological Procedures

Figure 16. Viral isolation from clinical specimens

conjugate was centrifuged at 2×10^3 RPM for 10 minutes in an IEC tabletop centrifuge to remove any particulate contaminants.

Those clinical isolates exhibiting CPE characteristic of adenoviruses and unidentified by enterovirus microneutralization procedures underwent fluorescent antibody staining. HeLa cells were grown in 125-mm tissue culture tubes to about 50% confluence and subsequently were inoculated with 0.1 mL of the virus suspension. The tubes were observed daily for evidence of CPE. When 75% of the monolayer showed viral involvement, the tube was vortexed to remove infected cells. In the case of negative controls (uninfected cells), the cells were scraped off of the glass with a rubber policeman. The tubes were then centrifuged at 6×10^3 RPM in an IEC centrifuge for 10 minutes. The supernatant fluid was decanted and the pelleted cells were washed three times with 5 mL of phosphate buffered saline (PBS), pH 7.6. After the last centrifugation the PBS was carefully decanted and the cell pellet resuspended in a minimal volume of saline (0.1 mL). The cell suspension was placed on a microscope slide, allowed to air dry, and fixed in cold acetone (-20°C) for 10 minutes. At this point, slides could be stored at -70°C to await further processing.

After warming to room temperature, fixed cells were covered with 0.05 mL of a 1:5 dilution of the adenovirus-specific fluorescein conjugate. Slides were incubated in a moist chamber for 30 to 45 minutes followed by two 10-minute rinses in PBS and a final distilled water wash. Cells were scored for adenovirus antigen production by visually observing fluorescence using a Zeiss Model 18 microscope equipped with an epifluorescent illumination and a fluorescein isothiocyanate (FITC) filter set.

Electron Microscopy of Fecal Specimens

Electron microscopic (EM) examination of fecal material has been used to distinguish an increasing number of morphologically distinct viral agents which have been associated with gastrointestinal illness. The virus particle types observed by EM in illness stools include: adenovirus, astrovirus, calicivirus, coronavirus, Norwalk-like or ''small round structured'' virus, and rotavirus. Routine cell culture techniques cannot currently be used to isolate many of these agents and specific immunoassays are only capable of detecting antigenically related viruses. As these agents are frequently shed by infected individuals in large numbers (1 g of stool may contain 10^{10} rotavirus particles), they are detectable by relatively insensitive EM procedures. Although dependent on virus type, state of aggregation, adsorption to grids, background material, and other factors, it was considered that a suspension titer of approximately 10^6 particles/mL would be required for detection by EM.

Using a negative staining technique, the USEPA HERL-Cincinnati laboratory has detected a number of these viral agents in illness stool specimens by EM. This technique was also used to examine approximately 1/4 of the stool specimens from the LISS. These specimens, labeled with the donor's name and code number, were shipped by UTSA to the USEPA laboratory in Cincinnati at various intervals during the intensive health watch. The specimens were shipped in glass vials on dry ice, in insulated containers. Shipping time was generally less than 24 hours and samples were cold upon receipt. All specimens were stored frozen at -70°C until processed as follows:

1) The fecal specimen was thoroughly mixed with a glass rod or pipette.
2) A small amount was removed and enough distilled water added to give a slightly turbid suspension.
3) A drop of the turbid suspension was placed on a copper EM grid (carbon substrate) and allowed to stand 1 minute.
4) Excess sample was removed with filter paper and the grid rinsed with one or two drops of distilled water.
5) The grid was negatively stained with a drop of 2% phosphotungstic acid (PTA), pH 7. The excess stain was removed with filter paper.
6) After drying, the grid was examined at 80 Kv on a JEOL 100CX transmission electron microscope for the presence of virus particles.

The detection of fecal viruses by EM using the negative staining technique has previously been described by Flewett (1978) and more recently by Field (1982).

Specimens yielding a Norwalk-like virus identification were sent to Dr. N. R. Blacklow's laboratory at the University of Massachusetts for examination of Norwalk-virus antigen by RIA.

F. LABORATORY ANALYSIS OF ENVIRONMENTAL SAMPLES

Wastewater Samples

Microbiological screens--

Indicator bacteria--Indicator organisms enumerated include total coliforms, fecal coliforms, and fecal streptotocci. These bacterial groups were detected using membrane filtration procedures as specified in Standard Methods for the Examination of Water and Wastewater, 14th Edition (1975) with the following exceptions. Based on experiences at other field sites, fecal streptococci were isolated on M-Enterococcus agar instead of KF Streptococcus agar (Sagik et al., 1980) Fecal coliform plates were incubated for 3-4 hours at 35°C to allow resuscitation of injured organisms before overnight incubation at 44.5°C. Additionally, the standard plate count as outlined in Standard Methods was used to determine the levels of aerobic and facultatively anaerobic, heterotrophic bacteria in each sample. Results for all indicator bacteria represent the mean of triplicate platings.

Other bacteria--

a. Salmonella--Prior to March 23, 1981, Salmonella screening was accomplished by filtering a measured volume of wastewater through a diatomaceous earth (DE) plug as described in Standard Methods (1975). Portions of the DE plug as well as aliquots of wastewater ($\leq$25 mL) were placed in separate bottles of selenite and tetrathionate broths for enrichment at 35°C. Aliquots from the broths were streaked for isolated colonies onto brilliant green agar and incubated at 42°C.

In an attempt to improve detection sensitivity, an alternative procedure described by Kaper and associates (1977) was tested. As described above, portions of the DE plug (for volumes >25 mL) and aliquots of wastewater were placed in dulcitol broth and incubated at room temperature for 4 hours followed by incubation at 35°C for an additional 18 to 20 hours. An aliquot from each primary enrichment volume was transferred into selenite cystine broth and incubated for 24 hours at 42°C. Subsequent plating was as described above.

Characteristic colonies were counted and tested for oxidase reactivity. Oxidase-negative organisms were transferred to an appropriate biochemical test screen: triple sugar iron (TSI) agar and lysine-iron agar (LIA). Based on these results, presumptive Salmonellae were confirmed with commercially available polyvalent and group-specific antisera.

As shown by results presented in Table A-17 in Appendix A, the double enrichment procedure yielded better recoveries of Salmonella from Lubbock wastewater. On this basis, this procedure was selected to replace the standard selenite enrichment technique.

b. Shigella--A portion of a DE plug resulting from filtration of wastewater as described under procedures for Salmonella along with ≤25-mL portions of the unconcentrated wastewater were used for detection of Shigella. Each of these samples was added to a separate bottle of GN broth. After 18 to 24 hours of enrichment at 35°C, aliquots from the bottles were dilution-plated onto xylose-lysine-deoxycholate (XLD) agar and incubated at 35°C. Oxidase-negative colonies were inoculated to a biochemical screen utilizing TSI and motility-indole-ornithine (MIO) medium. Shigella isolates were confirmed using commercially available polyvalent and group-specific antisera.

c. Staphylococcus aureus--Aliquots of wastewater were spread-plated onto mannitol salt agar and incubated at 35°C. Typical colonies showing a yellow zone of mannitol fermentation were counted and identified by microscopic observation of Gram-positive cocci and by testing for coagulase activity.

d. Mycobacterium--Mycobacteria were assayed quantitatively by a procedure which almost totally suppresses sewage saprophytes while permitting recovery of most mycobacteria. The sample was treated for 20 to 30 minutes with 500 ppm of benzalkonium chloride (Zephiran), diluted and plated onto the surface of previously prepared plates of Middlebrook 7H11 agar plus OADC enrichment modified by the addition of 3 µg/mL of amphotericin B. Plates were incubated at 37°C in a CO_2 atmosphere and examined over a period of 1 month for the appearance of typical colonies of mycobacteria. Suspect colonies were identified by examination of stained (Ziehl-Neelsen) smears for acid-fast bacilli. Additionally, all nonchromogens were subcultured onto Lowenstein-Jensen tubed medium and subsequently tested for niacin production, a distinguishing characteristic of M. tuberculosis.

If the density of mycobacteria was low, a concentration procedure was employed to improve detection sensitivity. A 50-mL volume of Zephiran-treated samples was centrifuged at approximately 5,000 x g for 20 minutes.

The supernatant fluid was discarded, the pellet resuspended in 1.0 mL of phosphate-buffered saline, and this volume plated as described above.

e. Klebsiella--Appropriate aliquots of wastewater were dilution-plated in triplicate to eosin methylene blue (EMB) agar and incubated at 35°C. Mucoid colonies were counted and tested for an oxidase-negative reaction. Suspect *Klebsiella* isolates were identified by typical biochemical reactions in TSI and MIO media.

f. *Yersinia enterocolitica*--As the detection of this organism was inconsistent during baseline monitoring using either enrichment or direct plate procedures, comparative testing of alternative methods was completed as described below.

Lubbock wastewater (trickling filter composite) was used unseeded and seeded with approximately 1 x 10^4 cfu/mL of *Y. enterocolitica* ATCC 23715. The different variables tested included the following:

1) Plating media
    - a) Salmonella-Shigella agar (SS)
    - b) MacConkey agar (Mac)
    - c) Cellobiose arginine lysine agar (CAL)

2) Cold enrichment media
    - a) 0.067M phosphate-buffered saline, pH 7.6 (PBS)
    - b) PBS with 1% mannitol, pH 7.3 (PBS-Man)
    - c) 0.85% NaCl with 25 µg/mL potassium tellurite (NS-PT)

3) Sampling periods
    - a) Direct
    - b) 3 days
    - c) 7 days
    - d) 14 days
    - e) 21 days

4) Treatment of inocula
    - a) Untreated
    - b) Potassium hydroxide treatment (KOH-NaCl)

Portions (150 mL) of the unseeded and seeded wastewater were filtered through separate 1-g DE plugs. One third of each plug was placed into the respective enrichment medium. The enrichment media were incubated in a refrigerator at 4°C. The seeded and unseeded wastewaters were sampled prior to filtration and enrichment, immediately after filtration and placement into the enrichment media (i.e., ''zero time''), and after cold enrichment for 3, 7, 14 and 21 days. In each case, inocula for the plating media were untreated or treated by mixing 20 µL of sample with 0.1 mL of 0.5% KOH in 0.5% NaCl just prior to plating. The plates were streaked by the four quadrant plating method and incubated at 25°C for 48 hours. Characteristic colonies were identified using the API 20E system.

Results of the comparisons of procedures of recovery of Y. *enterocolitica* from the seeded and unseeded samples are shown in Tables A-18 and A-19 in Appendix A, respectively. A semiquantitative index of the numbers of this organism present was obtained by reporting the highest quadrant in which the organisms were isolated as discrete colonies. It was apparent from these results that Y. *enterocolitica* could readily be isolated from both the seeded and unseeded wastewater samples.

The cold enrichment medium (NS-PT) previously employed (Sonnenwirth, 1974) proved to be markedly inhibitory to the organism in both seeded and unseeded samples; however, both PBS and PBS-Man yielded Y. *enterocolitica* at the different sampling periods, particularly when the inocula were treated with KOH-NaCl. Y. *enterocolitica* was recovered from each of the plating media. However, the greatest percentage of isolates picked that proved to be Y. *enterocolitica* by the API 20E were from CAL. Colonies of the organism were very distinctive on CAL in contrast to Mac and SS agars.

Based on these results, Y. *enterocolitica* was detected by the following enrichment procedure beginning with samples collected on March 23-24, 1981. A measured amount of wastewater was filtered through a 1-g DE plug which was subsequently dispersed in PBS (50 mL). A volume was removed for plating at this time and after 3 days of incubation at 4°C. Plating volumes were treated with KOH-NaCl and plated onto CAL agar. Typical colonies were isolated after 48 hours of incubation at room temperature (22 to 25°C) and identified using API 20E and oxidase tests.

g. *Clostridium perfringens*--An MPN procedure was used to enumerate both vegetative and sporulated Clostridia. Prior to analysis, a portion of the wastewater was heated at 80°C for 30 minutes. Both this heat-shocked and the untreated sample were diluted appropriately in PBS and inoculated into three tubes of differential reinforced clostridia medium (DRCM) at each dilution. Following incubation at 35°C for 72 hours, a loopful of sample from each DRCM tube was transferred to litmus milk and subsequently examined for typical stormy fermentation to confirm the presence of C. *perfringens*. Organism densities were computed from the MPN tables in *Standard Methods* (1975).

An alternate membrane filtration (MF) procedure for the enumeration of C. *perfringens* as described by Bisson and Cabelli (1979) was evaluated in parallel with the MPN procedure described above. A volume of wastewater was filtered through a 0.45-µ membrane filter (Gelman GC-6) which was placed onto mCP agar containing cycloserine and polymyxin B sulfate as inhibitory agents. Plates were incubated anaerobically in the BBL Gas Pak system at 45°C for 18 to 24 hours. Sucrose positive, cellobiose negative (yellow colored) colonies were counted and tested for positive reactions for acid phosphatase and gelatinase. Further confirmation involved subculture to litmus milk with stormy fermentation followed by testing for lactose, mannose and sucrose (with gas production) fermentation and nonfermentation of cellobiose, mannitol and salicin. Additionally, Gram-positive rods were visualized from litmus milk cultures.

Results of parallel testing are presented in Table A-20 in Appendix A. The multiple tube technique detected a higher level of vegetative C. perfringens (nonheated sample) than the MF method in all of the samples analyzed. The MF method detected a higher level of sporulative C. perfringens (heated-treated sample) on two of four samples. This result could be attributed to the milder heat treatment process used in the MF method. Perhaps more importantly, the confirmation of C. perfringens by visualization of Gram-positive, nonmotile rods was nearly equivalent for both procedures.

Due to the nature of the MF technique, this procedure was used when larger volumes of samples were processed. Specifically, this MF technique was applied to the recovery of C. perfringens from selected aerosol samples during 1982. It should be noted, however, that results from the MPN and MF procedures should not be directly compared.

h. Campylobacter jejuni--Beginning with samples collectd in July 1981, an assay to allow the detection of C. jejuni in wastewater was included in the microbiological screen. Aliquots of wastewater were spread onto the surface of Campy-BAP agar plates supplied by San Antonio Biological Company. This medium consisted of brucella agar base with 5% sheep erythrocytes and vancomycin (10 mg/L), trimethoprim (5 mg/L), polymixin B (2500 I.U./L), amphotericin B (2 mg/L), and cephalothin (15 mg/L). Plates were incubated in a microaerophilic environment (Campy-PakII) for 48 hours at 37°C. Suspect colonies were subcultured to 5% sheep blood agar, incubated as before, and nonhemolytic reactions typical of C. jejuni were noted. Further tests for this organism included catalase production, oxidase production, growth in 1% glycine, lack of growth in 3.5% NaCl, sensitivity to nalidixic acid (30 μg disk) and darting motility as observed microscopically in wet mounts.

i. Candida albicans--Testing for this organism was initiated as part of wastewater screens in July 1981. Appropriate dilutions of wastewater were spread onto Sabouraud dextrose agar (SDA) supplemented with 200 μg/mL chloramphenicol. Plates were incubated at 37°C for 48 hours. Suspect colonies were subcultured onto SDA prior to confirmatory testing which consisted of positive germ tube formation in bovine serum, positive chlamydospore production on cornmeal-Tween 80 agar, and assimilation of sucrose as the sole carbon source.

j. Fluorescent Pseudomonas sp--Aliquots of wastewater were spread-plated onto Cetrimide agar (DIFCO) and incubated at 35°C for 24 hours. Plates were then moved to room temperature for an additional 20-24 hours. Fluorescent colonies were counted while viewing plates under long-wave ultraviolet light using a Chromato-Vue cabinet (Ultra-Violet Products, Inc; San Gabriel, California).

k. Gram-negative enteric bacteria--Both oxidase-negative and oxidase-positive enteric bacteria including all members of the family Enterobacteriaceae were sought using the screening procedures diagrammed in Figure 17. Wastewater samples were diluted appropriately in sterile phosphate-buffered saline

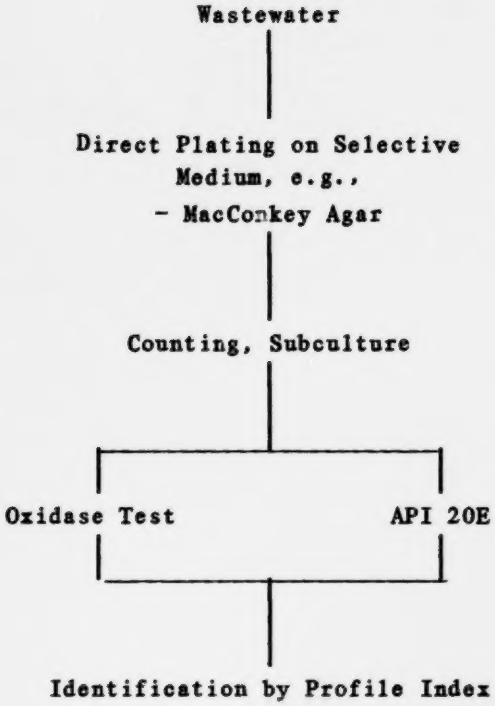

Wastewater
Direct Plating on Selective Medium, e.g.,
- MacConkey Agar
Counting, Subculture
Oxidase Test
API 20E
Identification by Profile Index

Figure 17. Isolation of Gram-negative enteric bacteria from wastewater

and spread over three plates per dilution on MacConkey agar. After incubation at 35°C for 24 hours, all colonies were counted and isolated at a dilution yielding a total of approximately 100 colonies over three plates. Discrete colonies were streaked onto quadrants of heart infusion agar plates to allow growth and confirmation of purity.

Subsequent identification involved oxidase testing and the use of API 20E identification strips. The API 20E system consists of a preset battery of 20 microtubes which allows the performance of 22 biochemical tests for the identification of 49 species/subspecies of Enterobacteriaceae and 38 group/species of other Gram-negative bacteria.

Bacteriophages--Coliphages indigenous to wastewater were assayed as plaque-forming units (pfu) using Escherichia coli K13 as the host organism. Tests in this laboratory have shown strain K13 to yield the highest coliphage titers when compared to other E. coli hosts. Appropriate volumes (0.1, 0.5, or 1.0 mL) of the wastewater and 0.5 mL of overnight culture of host cells were added to 3.5 mL of liquefied tryptose-phosphate soft agar and poured while warm (45°C) onto 100-mm petri dishes prepared with 10 mL of solidified tryptose-phosphate agar base layer. When firm, the plates were inverted and incubated at 35°C for approximately 18 hours prior to counting. For each sample, a minimum of five plates was used.

Human enteric viruses—During 1980, two concentration techniques were used in parallel for the recovery of human enteric viruses from wastewater samples. Both bentonite adsorption and organic flocculation were used to concentrate indigenous viruses from the five effluent samples. This approach was deemed necessary due to the nature of the wastewater entering the Lubbock treatment plant, i.e., both industrial and domestic wastes.

Positive viral recoveries were made consistently from the bentonite concentrates, while parallel assays of the organic flocculation concentrates were less successful due to toxicity and contamination. The standard bentonite concentration procedure performed adequately on both Lubbock and Wilson wastewater effluents. Viral concentration efficiencies based on the recovery of poliovirus 1 (Chat) were relatively consistent with a mean of 67 ± 26% for Lubbock wastewater (14 samples) and 58 ± 16% for Wilson effluent (14 samples) collected during 1980 and 1981. Concentrated volumes were suitable for both plaque and tube culture assay.

In addition, the bentonite adsorption technique has isolated a wide spectrum of enteroviruses as shown in Table A-21 in Appendix A. It should be noted, however, that this concentration technique was not expected to recover either reoviruses or adenoviruses.

Based on these observations, the bentonite adsorption procedure as described below was used as the sole viral concentration technique for wastewater effluents.

For detection of human enteric viruses, a maximum of 4 L of treated wastewater was concentrated in the laboratory using a standard bentonite adsorption technique (Moore et al., 1979). Briefly, wastewater was placed in a vessel of convenient size and 100 mg/L of expanded bentonite was added along with sufficient $CaCl_2$ to bring the wastewater to approximately 0.01 M. The pH of the sample was adjusted to 6.0 with HCl, and it was mixed for 30 minutes. After mixing, the virus-solids-bentonite complex was sedimented by low speed centrifugation. Tryptose-phosphate broth (TPB) was added to the pellet to facilitate viral elution at a ratio of 10 to 15 mL of TPB per liter of sample concentrated. Elution was accomplished by sonicating the TPB-solids-virus suspension for 5 minutes in an ice bath. The suspension was separated by centrifugation (8,000 x g), and the supernatant fluid containing the eluted virions was held at -76°C for assay.

Indigenous enteric viruses were enumerated by plaque assay on selected cell monolayers. Testing conducted as part of the wastewater pathogen screens during 1980 led to the selection of HeLa and RD cell lines for viral recovery from environmental samples. Data presented in Tables A-21 and in A-22 in Appendix A substantiate the choice of these cells in a complementary assay system. In this laboratory HeLa cells recover the greatest variety of enteric viruses. During baseline monitoring, the RD cell line showed a preferential recovery of echoviruses, even in the presence of polioviruses and Coxsackieviruses as evidenced by results from Lubbock-1 and Lubbock-2 samples (see Table A-22 in Appendix A). Additional testing has shown that echoviruses can be isolated as plaques on the RD cell line. To further enhance the recovery of a broad spectrum of enteroviruses, a portion of each concentrated volume was neutralized for all three poliovirus serotypes prior to the assay to avoid overgrowth and interference.

Beginning in January 1981, the assay matrix shown in Table A-23 in Appendix A was used. To optimize the use of neutralizing antisera, total enteroviruses were assayed first on HeLa monolayers. Based on the results of this analysis, subsequent assays using poliovirus antisera were completed.

At the time of inoculation each series of ten (or five) plates was assigned a number (1 through 5 or 10, as appropriate). A random ranking of numbers was created for each assay system by lottery draw. The numbers were recorded on the assay sheet in the order in which they were pulled. After the appropriate incubation period, pfu were counted on those plates yielding countable plaques. Plaques were picked for confirmation and storage from plates at the dilution which allowed the best separation of pfu and reflected the viral level to be reported. Selection of pfu from plates followed the previously recorded order. Thus, if the ranked order of RD plates (undiluted sample) was 3, 1, 8, 2, etc., all plaques on plate 3 were picked followed by plates 1, 8, etc., until the desired maximum number of pfu were acquired. If one plate was unacceptable due to overlap of pfu or contamination, the next listed plate was used. The following guidelines were followed in picking plaques for confirmation and possible future identification: 25 pfu from the unaltered HeLa assay and 15 pfu from each assay of polio-neutralized sample on HeLa and RD cells. In those cases when fewer than the specified number of viral plaques were evident, all pfu were picked. All pfu were confirmed by passage in the homologous cell line, logged, and frozen at -76°C if viral identification was indicated.

Poliovirus neutralization was done using commercially available rabbit antisera (M.A. Bioproducts). During 1981 the commercial supply of specific poliovirus antisera was discontinued. Subsequently, lyophilized monkey or equine sera were obtained from the National Institutes of Health for use in the poliovirus neutralization assays. Each lot of antisera was used at a level which had previously demonstrated at least a 3.0 $\log_{10}$ plaque reduction of homologous laboratory strains of poliovirus. Representative data showing poliovirus neutralization by monospecific antiserum is presented in Table A-24 in Appendix A. Sample and diluted antisera against polio 1, 2 and 3 were mixed, incubated in a 37°C water bath for 30 minutes, and plated.

The generalized procedure for plaque assay consisted of inoculating confluent cell monolayers grown in 100-mm plates with 1.0 mL of sample. After a 60-minute infection period, monolayers were overlaid with an agar-based Eagle's minimal essential medium containing bovine serum and antibiotics. Infected plates were held at 37°C in a 5% CO_2 humidified incubator. Two to three days post-infection, a second overlay containing 30 μg/mL of neutral red was placed on each plate. Plates were read on each succeeding day and scored for plaques through 5 days.

Possible viral isolates were picked from areas exhibiting characteristic cytopathic effect (CPE) based on microscopic examination of the stained monolayer. The removal of plaque-like areas was accomplished by first removing the second overlay above the area of CPE. Agar overlaying the entire plaque was aseptically collected using a microspatula. The sample was placed in 0.5 mL of medium 199 containing antibiotics and held at -76°C until confirmation.

Confirmation of potential viral isolates was performed in homologous tube culture systems. Culture tubes were grown out to 50 to 75% confluence and inoculated with 0.2 mL of sample. After 48 hours of incubation at 37°C, tubes were observed daily for evidence of CPE. When characteristic CPE was observed, the sample was removed and frozen at -76°C for viral identification. After 7 days, all samples not showing CPE were harvested and blind-passaged. Those isolates that demonstrated CPE after a second passage also were also reported as viruses (pfu).

Viral isolates were identified using the Lim Benyesh-Melnick pools for typing enteroviruses (Pools A-H and J-P) in a microneutralization procedure.

Physical-chemical analysis--Total suspended solids (TSS), total volatile suspended solids (TVSS), and total organic carbon (TOC) were analyzed following procedures outlined in Standard Methods (1975). Values reported are the mean of triplicate analysis for each parameter.

Routine wastewater samples--

Routine wastewater samples were intended to allow a determination of potential exposure of the study population when the wastewater was used in irrigation. Samples were cooled to 4°C in wet ice and shipped to UTSA at that temperature for analysis.

The routine wastewater samples were analyzed for total and fecal coliforms, coliphage, fecal streptococci, mycobacteria, enteric viruses, TSS, TVSS, and TOC. Analytical procedures were those described above under ''Microbiological Screens.''

Enterovirus Identification Samples--

Composite samples were collected from the Lubbock treatment plant trickling filter effluent or from effluent from the pipeline at the irrigation site (when available) and from the Wilson Imhoff tank effluent. Samples were cooled to 4°C and shipped to UTSA/UTA. The enterovirus identification samples were analyzed for human enteric viruses, fecal coliform, TSS, TVSS, and TOC following the procedures described above under ''Microbiological Screens.'' Plaques were picked, confirmed and up to 50 viral isolates per sample were frozen at -76°C for future identification. Within the limits of the assay systems employed, the analysis of these samples allowed the determination of enterovirus types present in the sprayed wastewater and circulating within the Wilson population.

Limited Bacterial Screen Samples-

Composite samples of Lubbock trickling filter effluent (or when available pipeline flow) were collected and shipped to UTSA as part of the enterovirus identification samples described above. In addition to physical-chemical analyses, the following potential microbiological pathogens were sought using procedures described under ''Microbiological Screens'': Salmonella, Shigella, Yersinia, Staphylococcus aureus, and Klebsiella-like organisms. On March 23, 1981, both Campylobacter jejuni and Candida albicans were added to this list of pathogenic organisms following methods described above. Beginning June 29, 1982, fluorescent Pseudomonas sp. was substituted for S. aureus. As part of an effort to characterize Wilson wastewater, the same limited bacterial evaluation screen covering these seven organisms was initiated on Imhoff tank effluent beginning in July 1981. The occurrence of selected organisms with human pahtogenic potential in wastewater destined for irrigation can thus be documented.

Legionella Samples--

Wastewater from the Lubbock sewage treatment plant was piped to three reservoirs located on the Hancock site and used for spray irrigation either directly or from these reservoirs. A total of nine separate wastewater samples were received by the University of Illinois during 1982. Five of these samples (one trickling filter effluent sample from March; three pipeline effluent samples from February, March and June; and one reservoir sample from June) were processed and inoculated into guinea pigs. Two samples (pipeline effluent and reservoir samples from July) were examined by direct fluorescent antibody (DFA) techniques for Legionella antigen. The two remaining samples (both reservoir samples from August) were not tested.

Complete testing for Legionella-group agents involved tenfold concentration of wastewater samples by centrifugation. Aliquots of the samples were then examined by DFA using available conjugates and diluted (serial tenfold) for total bacterial counts using standard methods. The purpose of this latter step was to avoid ''overloading'' guinea pigs with more than 10^6 to 10^7 non-Legionella organisms and it was anticipated that samples would be diluted to this level. However, this concentration was generally found either in the tenfold concentrated or unconcentrated samples, making further dilution unnecessary. Guinea pigs were inoculated intraperitoneally with 1.0 mL of samples. Samples seeded with a standard amount of virulent L. pneumophila 1 were included as controls. Guinea pigs were observed daily and rectal temperatures recorded. Animals having a fever for two consecutive days were euthanized. A fever was defined as a 0.5°C increase in rectal temperature above preinoculation values. Since animals inoculated with this type of material would be expected to develop fevers unrelated to Legionella infection after inoculation, fever 3 days postinoculation was taken as a possible indication of a Legionella infection. All animals were euthanized on the seventh day postinoculation and were autopsied within hours of euthanization or dying. Sterile techniques were used to collect peritoneal exudates and spleens. Samples of these fluids or tissues were examined by DFA for Legionella and were inoculated onto a variety of non-selective and semiselective agar media. Potential Legionella colonies were passed on charcoal-yeast extract (CYE) agar. Second passage material was inoculated onto trypticase soy agar (TSA) plates. CYE colonies failing to grow on TSA were considered possible evidence of Legionella.

A number of attempts were made to isolate Legionella directly from wastewater samples. These included inoculation of samples onto plates of the semiselective medium BMPAα (Edelstein, 1981) which contained cefamandole, polymyxin B, anisomycin, an organic buffer, and α-ketoglutarate and pretreatment of samples with an acid buffer (pH 2.2) as described by Bopp and associates (1981) followed by inoculation onto BMPAα.

Aerosol Samples

The composite samples of sprayed wastewater taken during the microorganism aerosol runs were analyzed for the same microorganism groups and water quality measurements as the routine wastewater samples. The aerosol sampler fluids from the microorganism aerosol runs and background runs and the aerosol and wastewater samples from the quality assurance runs were assayed for fecal coliforms, coliphage, fecal streptococci, and mycobacteria or Clostridium perfringens. Assays for human enteric viruses were conducted on the wastewater and aerosol samples from the enterovirus runs. Procedures for the indicator bacteria, mycobacteria, C. perfringens, coliphages and human enteric viruses are described in ''Microbiological Screens.''

The aerosol concentration procedures for human enteric viruses described by Moore et al. (1979) was developed to be performed at a field site. Due to the relative proximity of the Wilson site and the reduced interval between sample collection and arrival at the laboratory, organic flocculation was evaluated as an alternate concentration procedure. It was considered probable that this procedure might provide higher viral recoveries.

Three enteric viruses were used in the procedure developement and comparison testing: poliovirus 1, coxsackievirus B3 and echovirus 6. These viruses were differentiated by using two cell lines and monospecific antiserum in the following combinations. To determine poliovirus 1 titers the sample was neutralized for coxsackievirus B3 and assayed on HeLa cells. Coxsackievirus B3 and echovirus 6 were assayed from samples treated with poliovirus 1 antisera and titrated on HeLa and RD cells, respectively (echovirus 6 will not plaque on HeLa cells; likewise coxsackievirus B3 will not plaque on RD cells). This assay scheme allowed all three viruses to be detected in one sample.

Typically, organic flocculation is performed by adding organics (beef extract) to a sample. These organics are precipitated out of solution when the pH is lowered to approximately 3.5. Virions are entrapped in the organic floc and removed by centrifugation. The amount of organics present in a solution frequently dictates viral recovery rates; therefore, experiments were performed to determine the optimal amount of beef extract that should be added to the sampler fluid (BHI + 0.1% Tween 80).

Poliovirus 1, coxsackievirus B3 and echovirus 6 were added to three liters of BHI + 0.1% Tween 80 to give a final concentration of approximately 10 to 100 pfu/mL and mixed for 15 minutes. Ten mL of the sample were removed to establish actual input titers and the remaining sample was divided into 500-mL test volumes. Beef extract was added, resulting in final concentrations of 0%, 1%, 2% and 3%. The pH of each aliquot was adjusted to 3.5 by the dropwise addition of 1N HCl. The samples then were mixed for 30 minutes and centrifuged for 10 minutes at 8000 x g. After the supernatant fluid was decanted, each pellet was resuspended in 10 mL of 0.15M Na_2HPO_4 (pH 9.0), and subsequently the pH was adjusted to 7.0. The final volume was measured and the sample assayed as previously described. For comparative testing, a 500-mL aliquot of seeded sampler fluid was concentrated by two-phase separation as described by Moore et al. (1979).

Results shown in Table A-25 in Appendix A demonstrate that the addition of 2% beef extract provided optimal recovery when compared to the other beef extract concentrations evaluated. Organic flocculation using 2% beef extract also consistently outperformed two-phase separation, especially in the recovery of echovirus 6. Therefore, the following protocol was adopted for the detection of viruses in aerosols.

The total volume of BHI + 0.1% Tween 80 from an aerosol run was measured and 100 mL of the sample removed for routine organism determinations. The amount of beef extract added to the sample was calculated on the basis of total volume minus 100 mL. The beef extract was added to a final concentration of 2% and mixed until the beef extract went into solution. The pH of the sample was then lowered to 3.5 with 1N HCl. After 30 minutes of mixing the organic floc was recovered by centrifugation at 8000 x g for 10 minutes. The pellet was resuspended in 140 mL of 0.15M Na_2HPO_4 (pH 9.0). The pH of the final eluate was adjusted to 7 and subsequently split into two equal portions, one to be assayed on HeLa cells and the other on RD cells. Prior to being assayed, the sample was treated with chloroform to reduce bacterial and fungal contamination.

Plaque assay conditions and viral confirmation and identification utilized the protocols described under ''Microbiological Screens.''

Fly Samples

An effort was made to isolate enteric bacteria and viruses from houseflies trapped at the farmhouses and at the effluent ponds. The insects were processed as outlined in Figure 18. The clinical bacteriology and virology procedures described previously were followed.

Drinking Water Samples

Indicator Bacteria--

Total coliforms, fecal coliforms, and fecal streptococci were enumerated using membrane filtration techniques described in Microbiological Methods for Monitoring the Environment (USEPA, 1978). Total coliform bacteria were assayed on M-Endo agar; fecal coliform, on absorbent pads saturated with M-FC broth; and fecal streptococci, on KF streptococcus agar.

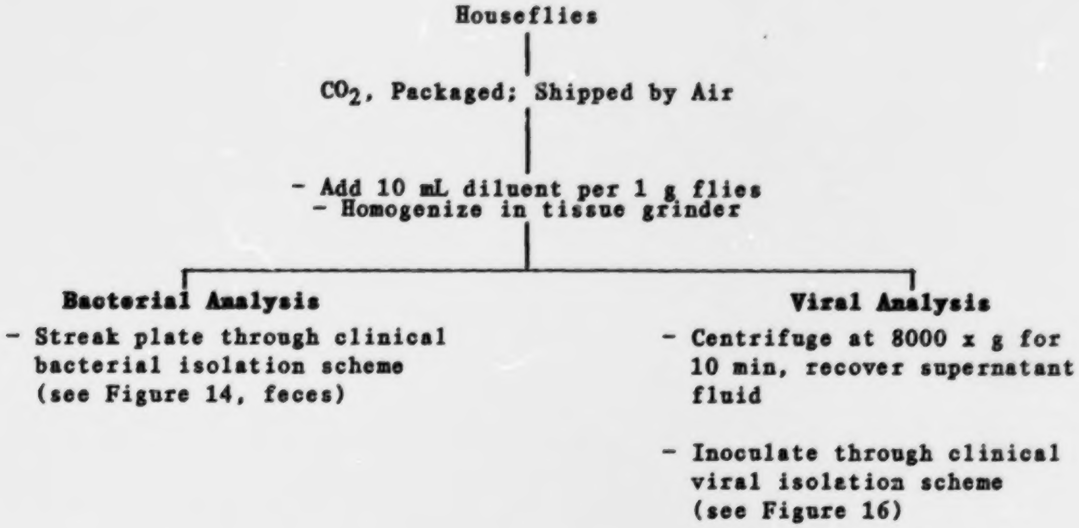

Houseflies
CO_2, Packaged; Shipped by Air
- Add 10 mL diluent per 1 g flies
- Homogenize in tissue grinder
Bacterial Analysis
- Streak plate through clinical bacterial isolation scheme (see Figure 14, feces)
Viral Analysis
- Centrifuge at 8000 x g for 10 min, recover supernatant fluid
- Inoculate through clinical viral isolation scheme (see Figure 16)

Figure 18 Analyses of insect vectors

Salmonella--

The presence of Salmonella was determined following procedures described in Microbiological Methods (1978) and Kaper et al. (1977). Organisms were recovered by filtering sample aliquots through a membrane filter which was subsequently incubated in 50 mL of dulcitol broth enrichment medium for 4 hours at 25°C, followed by 20 hours at 35°C. One mL of this enrichment medium then was transferred to selective selenite cystine broth and incubated at 41.5°C for 24 hours. Aliquots from these selenite cultures were streaked onto brilliant green agar. Salmonella colonies, which appeared pink to white and opaque surrounded by a brilliant red zone were subcultured to BHI agar. A dense suspension of bacterial growth was prepared in phenolized saline on a slide. A drop of polyvalent (A-I) Salmonella antiserum was added to the cell suspension. Rapid cell agglutination was scored as a positive response for detection of Salmonella.

G. INFECTION EVENTS AND EPISODES

Bacterial Infection Event

A fecal donor was considered to be having a bacterial infection when an overt or opportunistic bacterial pathogen was isolated from a fecal specimen at or exceeding a specified semiquantitative level which might be associated with enteric disease. The levels equated with bacterial infection were:

Category 1	any isolate of a major enteric bacterial pathogen (i.e., Salmonella or Shigella species, Campylobacter jejuni, or Yersinia enterocolitica);
Category 2	isolation at the heavy level of a possibly significant opportunistic pathogen (i.e., API Group I, Candida albicans, Chromobacterium, Citrobacter, Klebsiella, Morganella, Proteus, Providencia, Serratia, and Staphylococcus aureus);
Category 3	isolation at the moderate or heavy level of selected organisms found to be uncommon in feces but prominent in the sprayed wastewater (i.e., Aeromonas hydrophila and the fluorescent Pseudomonas group: P. aeruginosa, P. fluorescens, and P. putida).

The infected donor was considered to have had an infection event since donation of the prior fecal specimen in the series when the level of the organism in the prior specimen had been:

1) negative, for major enteric pathogens,
2) negative to light, for possibly significant opportunistic pathogens,
3) negative to light, for organisms prominent in the wastewater.

These criteria for a bacterial infection and for a bacterial infection event are summarized for all three bacterial pathogen categories in Table 10.

It was of primary interest to determine the bacterial infection status of a routine fecal specimen donor in relation to a period of irrigation. Routine specimens were collected from designated donors in scheduled weeks before, during and near the end of each irrigation period (see Figure 2), usually at intervals of about 6 and 4 weeks, respectively. Thus, the onsets of bacterial infection events could be temporally related to wastewater irrigation periods. When the change in infection status occurred between

TABLE 10. BACTERIAL INFECTION CRITERIA

Agent	Donor infected	Infection event
		(FROM) —> (TO)
Overt Pathogens	+ (E,VL,L,M,H)	- —> +
Salmonella		
Shigella		
Yersinia enterocolitica		
Campylobacter jejuni		
Possibly Significant Opportunistic Pathogens	H	-,E,VL,L —> H
API Group I		
Candida albicans		
Chromobacterium		
Citrobacter		
Klebsiella		
Morganella		
Proteus		
Providencia		
Serratia		
Staphylococcus aureus		
Opportunistic Pathogens Uncommon in Feces but Prominent in Wastewater	M,H	-,E,VL,L —> M,H
Aeromonas hydrophila		
Fluorescent Pseudomonas		

Semiquantitative Levels:

- - negative
E - enrichment
VL - very light (1-10 colonies on plate)
L - light (growth in first quadrant)
M - moderate (growth on first 2 quadrants)
H - heavy (growth on 3 or 4 quadrants)

the two specimens donated during an irrigation period, onset occurred in the interim (i.e., during the irrigation period). When the change in infection status occurred in consecutive specimens donated before and during the irrigation period, it was uncertain whether onset occurred after irrigation commenced. When a bacterial agent was not recovered at a level equated with infection in either routine fecal specimen provided during an irrigation period, the donor was considered to have experienced no infection events by the agent during the observation period preceding and spanning the collection dates of the consecutive specimens.

Viral Infection Event

A viral infection event was defined as the detection of a specific virus by laboratory cultivation or by EM examination in the second and not the first of paired fecal specimens from the same person. Subsequent recovery of the same virus in a specimen from the same individual would be a new event if more than 6 weeks elapsed between sequential recoveries. Detection of a virus in the first of serial specimens was also considered a viral infection event. Viral infection status was correlated with an irrigation period in the same manner as bacterial infection status.

Serological Infection Event (Serological Conversion)

A serological conversion (''seroconversion'') was defined as a fourfold or greater rise in agent-specific antibody titer in successive sera from one individual that were tested simultaneously. Since successive sera from 1982 and 1983 spanned an irrigation period and several additional months (see Figure 2), it was not possible to determine if the onset of serologically detected infection events was during the irrigation period.

Identification of Infection Episodes

An infection episode was defined as the observation in the study population of a number of similar infection events (either serologically, microbiologically, or clinically) within a restricted interval of time. The minimum number of infections which constituted an infection episode was set by determining the number of infections that would be needed to reject the null hypothesis (of no association between infection status and wastewater exposure), assuming that all of the infections occurred in the high exposure group and no infections occurred in the low exposure group. Infection episodes were classified as exposure situations when the observation period corresponded to one or two major irrigation periods and when the causative agent was found (or could be presumed) to be present in the wastewater at that time. Infection episodes were classified as control situations when the causative agent could not survive in wastewater (i.e., influenza A) or when the episode preceded the start of irrigation. Each exposure and control infection episode was statistically analyzed for association with wastewater aerosol exposure.

To express these ideas more precisely, consider a specified set of similar agents whose infection events were to be analyzed as a group. Also consider a specified time interval over which the infection events were observed (an interval which usually spanned a single irrigation season). The infection status of each monitored specimen donor (i.e., whether newly infected or not infected by any agent in the group) was observed over the specified time interval. Denote by X_2 the number of infection events in the high exposure group of size n_2 due to a given agent (group) and let X_1 be the number of infection events due to the same agent (group) in the low exposure group. A ''high'' rate of infections is said to occur when a sufficient number of infection events ($X_1 + X_2 \geq b_o$) were detected in the entire monitored study population. The number b_o was chosen so if all these infection events had occurred in the high exposure group and none in the low exposure group, the appropriate statistical test would reject the null hypothesis of no association between infection status and wastewater exposure. The critical number b_o of infection events in the study population sufficient to constitute an infection episode is given in Table 11 for realistic values of n_1 and n_2 for the fecal donor sample ($n \approx 100$) and for the blood donor sample ($n \approx 300$). A significance level $\alpha = 0.05$ was chosen if the agent(s) were recovered from the sprayed wastewater during the specified irrigation season (or could be inferred from the available wastewater data to have been present, with likelihood exceeding 0.95). A significance level $\alpha = 0.01$ was chosen if the agent(s) were not recovered from the wastewater sprayed at that time.

TABLE 11. NUMBER OF CASES (b_o) REQUIRED FOR REJECTION OF $P_1 = P_2$ IN FAVOR OF $P_1 < P_2$ IF ALL CASES OCCUR IN THE SMALLER GROUP (n_2) AND NONE OCCUR IN THE LARGER GROUP (n_1)

				α					
n	r	n_1	n_2	0.01	0.025	0.05	0.10	0.15	0.20
100	1	50	50	6	5	5	4	3	3
	0.5	67	33	4	4	3	3	2	2
	0.2	83	17	3	2	2	2	2	1
	0.1	91	9	2	2	2	1	1	1
300	1	150	150	7	6	5	4	3	3
	0.5	200	100	5	4	3	3	2	2
	0.2	250	50	3	3	2	2	2	1
	0.1	273	27	2	2	2	1	1	1

α - calculated using Fisher's exact test
$r = n_2/n_1$

Based on these criteria, the minimum number of infection events required to constitute an infection episode was determined (see Table 12) to be:

3 for agents detected in the blood donor or fecal donor populations and also recovered in the sprayed wastewater,

4 for agents observed in the fecal donor population but not recovered in the sprayed wastewater,

5 for agents detected in the blood donor population but not recovered in the sprayed wastewater.

TABLE 12. INFECTION EPISODE CRITERIA

Subpopulation	Agent recovered from sprayed wastewater?	α	Required number of infection events
Blood donor (n=300)	Yes	0.05	≥3
	No	0.01	≥5
Fecal donor (n=100)	Yes	0.05	≥3
	No	0.01	≥4

The periods of observation of infection episodes were chosen to coincide as closely as possible with the major irrigation periods:

		Period of observation	
Irrigation season	Period of irrigation	Fecal specimen series	Paired sera
1. Spring 1982	2-16 to 4-30-82	1-4 to 4-2-82	1-4 to 6-9-82
2. Summer 1982	7-21 to 9-17-82	6-7 to 9-17-82	6-7 to 12-10-82
3. Spring 1983	2-15 to 4-30-83	1-31 to 4-22-83	12-6-82 to 6-10-83
4. Summer 1983	6-29 to 9-20-83	6-6 to 8-19-83	6-6 to 10-13-83
5. 1982	2-16 to 4-30 and 7-21 to 9-17-82		1-4 to 12-10-82
6. 1983	2-15 to 4-30 and 6-29 to 9-20-83		12-6-82 to 10-13-83

Periods of serological observation which spanned the entire 1982 irrigation period (i.e., Jan 4-Dec 10, 1982) and the entire 1983 irrigation period (i.e., Dec 6, 1982-Oct 13, 1983) were employed to utilize serologic infection events whose time of occurrence could be ascribed to an annual period but not to a semiannual period. Baseline infection episodes occurring before irrigation commenced were also defined and analyzed with respect to the subsequent spring 1982 exposure grouping in order to investigate unmeasured potential risk factors which might be associated with the wastewater exposure measure in the study population and hence produce spurious associations with exposure in the infection episodes after irrigation commenced.

Infection episodes were defined for specific single agents whenever sufficient infection events to the agent occurred, as indicated in Table 12. Infection episodes were also defined to interpretable groups of specific agents when the infection events were scattered among the agents in the group.

The dependent variable defined for each observed participant in every infection episode was the number of infection events to the agent (or agent group) detected in the participant during the period of observation. A participant was seldom observed to experience more than one infection event to the agent (group) during the observation period of an infection episode, except in the serologic infection episodes to grouped agents over a 1-year observation period. To permit use of sensitive statistical methods requiring that the dependent variable only assume the values 0 or 1, all multiple infection events were treated as single infection events in most statistical analyses performed.

The convention used to construct the names of the dependent variables of all observed infection episodes is presented in Table 13. The dependent variable name is used throughout Sections 5 and 6 of this report to specify the infection episode when descriptions, statistical results and findings regarding the episode are presented.

The clinical (C) bacterial and viral agents and agent groups for which infection episodes were identified from series of monthly routine fecal specimens were:

KLB	*Klebsiella*
OOB	Other possibly significant Opportunistic Bacteria (all Category 2 opportunistic bacterial pathogens except *Klebsiella*)
PBW	Prominent Bacteria in Wastewater (Category 3 organisms which were uncommon in feces but prominent in the sprayed wastewater: *Aeromonas hydrophila* and the fluorescent *Pseudomonas* group)
VIR	all VIRal isolates (excluding adenoviruses and immunization-associated polioviruses). Adenovirus shedding is sporadic and may represent a prolonged latent infection. Poliovirus excretion following immunization is presumably not wastewater associated.
WWI	all WasteWater Isolates (all clinical isolates recovered from the sprayed wastewater during the irrigation period under observation)

For the all wastewater isolate (WWI) infection episodes, each bacterial and viral pathogen was listed that was isolated from any pipeline or reservoir wastewater sample taken during the irrigation period. The agents from the list which were also recovered from clinical specimens during the same irrigation season are presented in Table 14.

When the pair of fecal specimens from which a clinical infection event was identified were both obtained between the start and finish of an irrigation period, the onset of the infection event was clearly during the irrigation period. However, when the first fecal specimen of the pair preceded the start of the irrigation period, the infection event onset may have preceded irrigation (and hence been unrelated to wastewater). Thus, whenever there were sufficient infection events, a dependent variable was defined and the statistical analysis was performed both excluding (X variable, see position 6 in Table 13) and including (W variable) the fecal donors whose infection event onset may have preceded the irrigation period. In the statistical analysis, the newly infected donors were contrasted with fecal donors who were not infected by the agent (group) over the whole period of observation of the infection episode. A list of the clinical infection

TABLE 13. INFECTION EPISODE DEPENDENT VARIABLE[a] NAME KEY

Position	Information	Values and interpretation	
1	Method of detecting infections	C	clinical (bacteriologic or virologic) analysis of routine fecal specimens
		S	serologic analysis of blood specimens
2-4	Agent (group)	Clinical agent groups	
		KLB	Klebsiella
		OOB	other (non-Klebsiella) opportunistic bacteria
		PBW	prominent bacteria in wastewater
		WWI	all isolates from wastewater
		VIR	all viruses (excluding adeno and immunization polio)
		Serologic agent groups	
		AD3	adeno 3
		AD5	adeno 5
		AD7	adeno 7
		CB2	coxsackie B2
		CB4	coxsackie B4
		CB5	coxsackie B5
		E01	echo 1
		E03	echo 3
		E05	echo 5
		E09	echo 9
		E11	echo 11
		E17	echo 17
		E19	echo 19
		E20	echo 20
		E24	echo 24
		PL1	polio 1
		PL2	polio 2
		PL3	polio 3
		SNV	all serum neutralization-tested viruses, except polioviruses
		POR	sporadic serum neutralization viruses (too few to be a distinct infection episode)
		WWV	all viruses recovered from wastewater
		RE1	reo 1
		RE2	reo 2
		ROT	rotavirus
		LEG	Legionella
		INA	influenza a

continued...

TABLE 13 (CONT'D)

Position	Information	Values and interpretation	
5	Period of observation	0	baseline
		1	Spring 1982
		2	Summer 1982
		3	Spring 1983
		4	Summer 1983
		5	1982
		6	1983
		7-9	nonstandard periods
6 (clinical only)		X	onset of all infection events during irrigation period
		W	includes infection events whose onset may have preceded the irrigation period

a Value of each dependent variable = number of infection events to agent (Pos. 2-4) observed in participant by method (Pos. 1) in time interval (Pos. 5). The values of each dependent variable for each observed participant was collapsed to 0 = not infected or 1 = newly infected for all statistical analyses (i.e., multiple infection events were ignored).

TABLE 14. AGENTS COMPRISING CLINICAL WWI EPISODE BY SEASON: WASTEWATER ISOLATES RECOVERED[a] IN ROUTINE FECAL SPECIMENS DURING SAME IRRIGATION PERIOD

	Number of donors infected, by irrigation period			
Agent	CWWI1W Spring 1982	CWWI2W Summer 1982	CWWI3W Spring 1983	CWWI4W Summer 1983
Klebsiella pneumoniae	2	12	1	11
Klebsiella oxytoca		1	1	1
Aeromonas hydrophila				2
Fluorescent Pseudomonas group	3	4	2	7
Poliovirus 1	4			
Poliovirus 2		1		
Poliovirus 3	1			
Coxsackievirus B4		1		
Coxsackievirus B5		3		1
Echovirus 17	2			
Echovirus 27	1			

a Recovered at levels defining an infection event.

episodes which were observed, defined and submitted to statistical analysis is presented later in Table 97.

The serological (S) agents and agent groups for which infection episodes were identified from simultaneously tested paired sera were:

AD3	ADenovirus 3
AD5	ADenovirus 5
AD7	ADenovirus 7
CB2	Coxsackievirus B2
CB4	Coxsackievirus B4
CB5	Coxsackievirus B5
E01	Echovirus 1
E03	Echovirus 3
E09	Echovirus 9
E11	Echovirus 11
E19	Echovirus 19
E20	Echovirus 20
E24	Echovirus 24
PL1	PoLio 1
PL2	PoLio 2
PL3	PoLio 3
SNV	all Serum Neutralization-tested Viruses except polioviruses (serologic agents listed above plus echoviruses 5 and 17)
POR	sPORadic serum neutralization viruses (consists of all SNV agents for which too few infection events occurred during the period of observation to constitute a distinct infection episode). Since wastewater contains many infectious agents, a sporadic episode to a variety of agents might be the most subtle effect of wastewater exposure.
WWV	all WasteWater Viruses (all SNV agents recovered from the wastewater sprayed during the period of observation)
RE1	REovirus 1
RE2	REovirus 2
ROT	ROTavirus (tested primarily in children)
LEG	Legionella
INA	INfluenza A (An epidemiologic control agent since influenza A viruses do not survive in the intestinal tract or in wastewater.)

Some donors experienced more than one infection event during a serologic infection episode. This occurred when the period of observation spanned three or more blood collection periods (allowing detection of multiple infections to the same agent) or when the infection episode involved a group of agents (allowing infections to several agents in the group). The following guidelines were used to determine the value of the dependent variable for a participant for each of the serologic infection episodes:

- If a person experienced an infection by an agent during a given interval of time, the number of infection events observed was coded as the person's infection status. Infection events were counted and included in analysis of the infection episode even though that person may not have been observed (i.e., provided blood samples) during the entire interval of time.

- If no infection was observed in a person but he only provided a blood specimen for the first portion of the time interval of interest, then the infection status for that participant during that interval was coded as ''missing.''

- If no infection was observed, but a person only provided blood for the last portion of the time interval in question, the coding for that interval was dependent upon the person's initial titer for the partial interval. The interval was coded as having ''no infection'' if the person had either no detectable titer or had the lowest measurable titer for that agent. If the initial titer was higher than the lowest measurable titer, then infection status for the interval was coded as ''missing.''

- For the infection episodes to the agent groups SNV, POR and WWV, the seroconversion status of a donor may not have been determined for all agents in the group. If any infection events were observed, the number of infections experienced by that donor was used as the value of the dependent variable during the period of observation. When no infection events were observed, but the seroconversion status to some of the agents was not determined for that donor, the donor was excluded from analysis (i.e., infection status was coded as ''missing'').

A list of the serologic infection episodes which were observed, defined and submitted to statistical analysis is presented later in Tables 98 and 99.

Many of the infection episodes observed were not independent, primarily because one episode was a (partial) subset of another, either in time (e.g., episodes for seasons 1, 2 and 5) or in agent grouping (e.g., CB5 is a subset of WWV which is a subset of SNV). Jointly independent groups of infection episodes pertinent to comparing exposure and control situations were defined by classifying episodes by agent category (single and sporadic vs. grouped), situation (exposure vs. control) and observation period (single season vs. year). These criteria for the six mutually exclusive and jointly independent groups of episodes which were used are presented in Table 15.

TABLE 15. CLASSIFICATION CRITERIA FOR JOINTLY INDEPENDENT GROUPS OF INFECTION EPISODES

Jointly independent episode group	Criteria: Agent category	Criteria: Situation	Criteria: Observation period	Notes on episode selection priority
A	single or sporadic	exposure	single season (1,2,3,4)	a
B	single or sporadic	exposure	year (5,6)	
C	single or sporadic	control	all: baseline (0) and year (for influenza)	
D	grouped agents	exposure	single season (1,2,3,4)	a,b
E	grouped agents	exposure	year (5,6)	b
F	grouped agent	control	baseline (0)	

[a] For clinical infection episodes with both X and W dependent variables, the X variable was selected for membership in the jointly independent episode group since it was more applicable to wastewater irrigation inferences.

[b] When both WWV and SNV episodes were defined (with WWV infection events a subset of the SNV infection events), the WWV episode was selected for membership in the jointly independent episode group since the WWV episode was more applicable to wastewater irrigation inferences.

H. DATA MANAGEMENT

Data Processing and Verification

The information obtained from the health watch was stored by household and participant identification numbers on a Control Data Corp. mainframe computer at SwRI. The Scientific Information Retrieval (SIR) data base management system was chosen for the LISS data base due to its advanced programming features and its integration with statistical packages such as Biomedical Computer Programs (BMDP), Statistical Analysis System (SAS) and Statistical Package for the Social Sciences (SPSS).

Results obtained from UTSA (clinical specimen assays), UI (interviews, self-reported illness data and serologic assays) and EPA-HERL (electron microscopy) were keypunched, key-verified, and placed on the SwRI data base. After logical tests were performed, SIR-generated reports and error lists were sent to the investigator who had completed the data reporting forms for further verification and error resolution. The verified data were also visually inspected for reasonableness by the health watch manager and the project manager. Another method was implemented later in which serologic data were coded, keypunched, verified, and corrected at UI; the entire serologic data file was then placed on the SwRI data base. Computer-generated labels had been affixed to the container of each sample at each stage of processing and the label information had been coded along with the analytical result to further reduce the chance of error. An overview of the processing of each set of data is given in Table A-26 in Appendix A.

Data Base Structure and Use

The LISS data base was arranged into eight record types, which allowed logical groupings of related variables (see Table 16). Record 1 consisted of household-based variables. The key variable (sort identifier) of Record 1 was HHID, a three-digit household identification number. The first digit of HHID represented the zone in which the household was located; households were numbered consecutively within each zone.

Record 2 contained participant-based independent variables. The key variables of Record 2 were HHID and ID, a five-digit participant identification number. The first three digits of ID consisted of the household identification number (HHID). Adults living in a household were numbered consecutively from ___01, beginning with the head of household; children were numbered consecutively from ___11, beginning with the oldest child. Most of the variables in Records 1 and 2 were obtained from the recruitment and update interviews (Appendices B, C and D).

Record 3 contained variables describing fecal and throat culture samples. Microorganisms isolated and corresponding growth levels were stored in Record 4. Records 3 and 4 were separate to allow for multiple or no agents detected in a sample. Key variables in Records 3 and 4 included SAMPRD (the period of observation), ID, SPECTYP (the type of specimen analyzed) and in Record 4, ORGNSM (the type of organism isolated).

Record 5 consisted of self-reported illness data from the health watch. The sort variables in Record 5 were SAMPRD, ID, ILLNESS (the classification of a reported illness) and ILLNO (an illness repetition code). ILLNO was used to account for the same illness occurring more than once in the same sampling period. Inconsistencies in the Record 5 data are discussed in Section 5E.

Record 6 contained exposure data from the major irrigation periods. The key variables in Record 6 were ID and SEASON (a number from 1 to 4 corresponding to spring 1982, summer 1982, spring 1983 and summer 1983, respectively). Methods of exposure estimation and the major exposure variables were presented in Section 4C. Results from the Wilson restaurant patronage survey were placed in Record 6 because they had the same sort identifiers.

The variables in Record 7 were the results from serologic analysis of blood samples. ID and AGENT (the serologic agent tested) were the key variables in Record 7.

Record 8 contained the dependent variables from each infection episode used in statistical analyses. The key variable for Record 8 was ID. Construction of the dependent variables in Record 8 was explained in Section 4G. Record 8 variables were derived from variables in Records 3, 4 and 7.

A copy of the data base was also placed on the IBM computers at UI and EPA-HERL. To perform the data analyses, appropriate data files were abstracted from the data base and transmitted to the cognizant investigator.

TABLE 16. STRUCTURE OF LISS DATA BASE

Record type	Description of variables	Sort identifiers
1	Household independent variables (from recruitment and update questionnaires)	HHID (household ID)
2	Participant independent variables (from recruitment and update questionnaires) and annual exposure variables	a. HHID b. ID (participant ID)
3	Clinical specimen description, virology and electron microscopy results	a. SAMPRD (data collection period) b. ID c. SPECTYP (specimen type)
4	Microorganism isolations from clinical specimens	a. SAMPRD b. ID c. SPECTYP d. ORGNSM (organism)
5	Self-reported illness data	a. SAMPRD b. ID c. ILLNESS (illness category) d. ILLNO (illness repetition)
6	Exposure and restaurant variables	a. ID b. SEASON (irrigation period)
7	Serology data	a. ID b. AGENT (agent tested)
8	Dependent variables (number of infection events) for each infection episode, previous titer for serologic infection episodes	ID

Retrieval files were created from the SIR data base for use in statistical analyses via BMDP, SAS and SPSS. Because EPA-HERL did not have the SIR data base management system, UI generated SAS files of the eight record types comprising the data base which were transferred by tape to HERL.

I. QUALITY ASSURANCE

Health Data and Specimens

All completed household health diary forms which were forwarded by field representatives to UI on a biweekly basis were checked for completeness and coded for data entry. In order to achieve consistency in classification of illness information, all illnesses were coded according to a standardized list of illnesses and conditions. Telephone reports and written diaries were compared for discrepancies, and whenever possible, those discrepancies were resolved prior to submitting the coded diaries for data entry. Logic checks were written and forwarded to data management for additional checks of the health diary information. All discrepancies identified by the logic checks were resolved, and the recoded information was forwarded to data management for inclusion in the data base.

The health watch manager or one of the field representatives also supervised the labeling of all specimens received from the study population. This policy was necessary in order to avoid the problem of technicians misidentifying similarly named study participants. Computer-generated labels containing the participant's name and ID number were used to identify samples as well as to generate packing lists whenever specimens were shipped. A log was kept for all blood and fecal specimens that were received, so that an additional source of documentation was available in order to resolve discrepancies.

All activity and exposure information provided by participants was checked for completeness and accuracy by the field representatives or the health watch manager before the information was forwared to SwRI for coding and data entry. Logic checks were also written and forwarded to data management for use with the questionnaire information. All discrepancies identified by this method were resolved and recoded. In addition, the health watch manager reviewed each household and participant record in order to verify information for responses which could not be addressed by logic checks. This information, which included important variables such as sex, age, and occupation, was corrected whenever necessary and any missing information was obtained by contacting the household in question.

Aerosol Measurement Precision

Inspection of the microorganism aerosol density data showed considerable variation, even between paired samplers. This measurement variation may result from differences in many factors, including aerosol density fluctuation, sampler operating procedures, undetected sampler contamination, shipping difficulties (e.g., temperature above 4°C), analytical laboratory techniques, and random error.

Two quality assurance aerosol runs were conducted to investigate the amount and source of imprecision of the aerosol density measurements for each microorganism group. Nine samplers were operated 3 meters apart in a line at the same distance from a nozzle line, so that all samplers were theoretically sampling the same aerosol density. The 100 mL of sampler collection fluid was normally split at the laboratory into four 25-mL portions for the four microorganism assays. On the quality assurance runs, each sample was split in the field into 25-mL portions which were labeled for specific analyses. Three of the four portions were labeled for assay for the same microorganism group. Hence, portion variation, which reflected shipping and laboratory-related variation, could be subtracted from measurement variation to estimate the magnitude of sampling-related variation relative to shipping/laboratory variation.

The data from the quality assurance runs are presented in Table A-27 in Appendix A. The microorganism density in air determined from portions from the same sampler often exhibited less variation than the measurements from different samplers, but there were exceptions.

The precision of a sample of n determinations of the same true concentration can be measured by the coefficient of variation, which is the ratio of the unbiased sample standard deviation to the sample mean:

$$CV = \alpha_n\, s/\bar{x}$$

where $\bar{x}$ - sample mean = $\Sigma x/n$
s - sample standard deviation = $[\Sigma(x-\bar{x})^2/(n-1)]^{1/2}$
α_n - bias correction factor = $[(n-1)/2]^{1/2}\,\Gamma[(n-1)/2]/\Gamma(n/2)$

The bias correction factor α_n adjusts for the bias in the sample standard deviation s as an estimator of the population standard deviation σ. The values of α_n approach 1.0 as n increases: α_2=1.253, α_3=1.128, α_4=1.085, and α_5=1.064.

To investigate the consistency of measurement variation over the entire range of aerosol densities sampled in the field, measurement coefficients of variation were determined for all situations in which several samplers were theoretically sampling the same true density of microorganisms in air. These situations were the paired samplers at three locations on each microorganism run and the samplers assigned the same microorganism assay on a quality assurance run. Coefficients of variation were calculated when microorganisms were detected in at least one of the sampler assays, assuming assays in which no microorganisms were recovered had a value of half the detection limit. The coefficients of variation for microorganism run pairs in the same density range were averaged to yield a more stable estimate of the measurement variation.

The average measurement coefficients of variation throughout the density range sampled are presented for each microorganism group in Table A-28 in Appendix A. Because the standard deviation calculated from a small sample is very imprecise, the average coefficients of variation are quite variable over a microorganism's density range. However, there is no consistent pattern in the magnitude of the coefficient of variation with increasing aerosol density. Hence, average measurement coefficients of variation were determined over all sample sets, with the values 0.43 obtained for coliphage, 0.46 for fecal streptococci, 0.67 for fecal coliforms, 0.72 for Clostridium perfringens, and 0.81 for mycobacteria. Therefore, the precision standard deviation of the aerosol density measurements ranged from 43% of the measured value for coliphage to 81% of the measured value for mycobacteria.

An investigation of the relative magnitude of the various sources of the measurement variation was conducted based on the quality assurance runs. Portion coefficients of variation were determined for each run, where the values from different samplers were averaged. The assay result reported by the laboratory is an average, i.e., the total number of colonies or plaques counted in spreading aliquots of the sample or its serial dilutions over m plates (usually m=3 plates for fecal coliforms and fecal streptococci and m=25 plates for coliphage). To estimate laboratory sources of variation, an average aliquot coefficient of variation was calculated for all assays on each quality assurance run using the aliquot standard error $s/\sqrt{m}$ in place of the standard deviation to obtain a variability measure comparable to the measurement and portion coefficients of variation. Since variances of independent variables are additive, the variation attributable to field sources was estimated by subtracting the variance for shipping and laboratory sources from the measurement variance. Similarly the variation attributable to shipping sources was estimated from the portion and aliquot coefficients of variation.

Each of these coefficients of variation are presented in Table A-29 in Appendix A. While the aliquot variation estimates are quite stable, the variation attributed to other sources was highly variable due to the limited amount of quality assurance data. Although a much broader range of aerosol densities was sampled in comparison with the Pleasanton study (Johnson et al., 1980), the average measurement coefficients of variation determined in the two studies were similar for fecal coliforms, mycobacteria, and Clostridium perfringens. However, the LISS data for fecal streptococci and coliphage only exhibited about 60% as much measurement variation as in the Pleasanton study.

Laboratory Analysis

Enterovirus Serology--

Although the serum neutralization test is qualitatively reliable, the titers that are obtained when this test is used cannot be considered absolute. Different laboratories using different cell lines, different virus strains, or other slight variations in procedure, can produce different titer results for the same positive sera. Results can also be affected by the virus dose, the age of the tissue culture cells, slight changes in the pH, etc. Since the titers are known to vary significantly between tests, an infection was not reported in this study unless a fourfold or greater increase in titer could be demonstrated in simultaneously tested sera.

Since all of the sera from the study population could not be tested for antibody to a given agent in a single test, results from two or more tests, which were run at different times in the study, were used to detect fourfold or greater increases in titer. Whenever a possible fourfold increase was detected, the sera in question were retested in pairs. Since the initial screening results were used to determine which sera were selected for retesting, titer variability was a concern. Therefore, in addition to the usual quality control concerns in a serology laboratory, such as eliminating potential sources of contamination or interferences with test results, there were two additional goals for the enterovirus serology quality control: to limit the known sources of variability in the serum neutralization test, and to quantitate the reproducibility of the serum neutralization results. This was accomplished by increasing the number of controls to include six replicates of a human sera with a ''high'' level of antibody, and six replicates of a single human sera with a ''low'' or ''intermediate'' level of antibody to a given agent. The titers obtained from the replicate tests were used to calculate the geometric mean titer (GMT) and the titer reproducibility (TR) (Wood and Durham, 1980). This information, which can be found in Appendix O was used to determine the reliabilty, as well as the variability, of the results from any given test. With the exception of echoviruses 3 and 24, the between-test variability of the non-polio enterovirus control titers was within acceptable limits. Low virus dosage in the tests done to confirm fourfold titer increases in antibody to echoviruses 3 and 24 caused the control titers to be unusually high. However, the variabilty of the tests done as a part of the routine screening for antibody to these two agents were within acceptable limits.

Eighteen sera were forwarded to the University of Iowa Hygienic Laboratory (UIHL) to determine the antibody titers to poliovirus types 1-3. A listing of the results is presented in Table A-30 in Appendix A. Comparison of UI and UIHL results indicates that there was a fourfold or greater difference in Poliovirus 1 titers in only 12% of the cases. There was a fourfold or greater difference 23% of the time for Poliovirus types 2 and 3. The UI titers were generally lower than UIHL titers. If the assumption is made that the UIHL titers were ''correct,'' then the recommendations for immunization (which were based on the UI titers) may have included participants who were adequately protected. This situation is preferrable in that there was less chance of failing to immunize a susceptible participant.

All enterovirus serology was performed by UI personnel under the guidance and supervision of the Virology Laboratory section of the Illinois Deparment of Public Health (IDPH). In addition, all enterovirus controls were examined and verified by IDPH supervisors. The IDPH provided all necessary reagents, glassware, media preparation rooms, hoods, and environmental chambers for the enterovirus serology. IDPH routinely tested the distilled water as well as all new reagent lots for contamination and toxicity. Environmental chambers were continuously monitored for temperature variation, and the media preparation and hood rooms were checked for contamination on a routine basis.

All virus stocks were originally obtained by IDPH from either CDC or American Type Culture Collection (ATCC), and all subsequent passages were well documented. All tissue cultures provided by IDPH were similarly verifiable. In order to avoid potential labeling or contamination problems, fresh monospecific antisera from CDC was used to reidentify all of the stock enteroviruses which were used in this study.

Hepatitis A Serology--

Quality assurance for the determination of antibody to hepatitis A virus (anti-HAV) was that built into the HAVAB test system. This involved the use of both positive and negative controls provided by the test system manufacturer (Abbott Laboratories) and determining that only repeatably reactive specimens (minimally two tests conducted on separate days) were considered to be positive for anti-HAV by the HAVAB test. As a further control measure, each analytical series of 100 tests included two or three sera from participants whose HAVAB reactivity had been established previously.

Additional quality assurance programs were conducted between May 1981 and May 1983 at both UTSA and UI. A total of 267 sera (including all positive sera) were retested in the blind. Only four discrepancies (1.5%) were found in the retesting. All four discrepancies were found with sera previously found to be ''borderline'' positives that changed to ''borderline'' negatives in the retest. Given the variable nature of this test, the reproducibility of the HAVAB results was considered excellent.

Clinical Bacteriology--

Quality control in the Clinical Bacteriology Laboratory involved a program of internal monitoring, seeded unknowns, and replicate, split clinical specimens. Internal monitoring included testing each new batch of culture media, testing reagents and stains, and quality control of biochemical tests. In addition, the plating and enrichment media and biochemical test media were assigned expiration dates that prevented use beyond the point where consistent results could be obtained. Periodic seeded, unknown specimens ensured the proficiency of the laboratory in correctly identifying organisms and determining the levels of organisms in the specimens.

Selected fecal specimens were split and coded as unknowns for clinical analysis in April and May 1981. A listing of coded split samples (generated during preanalysis sample handling) was forwarded to the laboratory supervisor on a weekly basis. Results of this QA testing for clinical bacteriology are presented in Table A-31 in Appendix A and indicated a very successful program. Of the 22 split samples, total agreement on both isolate identification and quantitation was recorded on 14 specimens (64%). In all remaining samples, the variance between results of known and QA tests involved a difference of a single quadrant level of microorganism growth. For example, in handling specimen 55913 (period 108) as a split sample, *Escherichia coli* was reported as moderate and heavy, respectively, while *Enterobacter cloacae* was detected as no more than 10 colonies on one sample. The results in Table A-31 indicated excellent repeatability in the clinical bacteriology laboratory.

Results of additional quality assurance unknowns performed in November 1982 are shown in Table A-32 in Appendix A. The unknown samples were given to the technician as seeded autoclaved fecal specimens in buffered glycerol saline (from routine fecal specimens that had been preserved by freezing for use in quality assurance unknowns). Each of the four unknowns was correctly identified both with respect to identity of organisms and the level of seeding.

The concentration of organisms in feces represented by different levels of growth on MacConkey agar plates streaked by the four quadrant method is suggested by the results of Table A-33 in Appendix A. Each of the values represents laboratory reports on ''blind'' (unknown) samples seeded with known concentrations of three organisms. The unknown samples were given to the technician as buffered glycerol saline suspensions (E. coli) or seeded autoclaved fecal specimens in buffered glycerol saline (K. pneumoniae and P. aeruginosa).

A similar experiment was carried out for throat swabs as shown in Table A-34 in Appendix A. Four different organisms (Streptococcus pyogenes, E. coli, Enterobacter cloacae and K. pneumoniae), previously observed in some illness specimens, were separately diluted in Todd Hewitt broth. One mL portions of the dilutions were placed into tubes which then received sterile swabs. The coded samples were then given to the technician who processed the samples as if they were throat swabs (see Figure 15 and associated discussion for details of analysis of throat swabs). Results are reported as levels of growth on replicate plates of blood agar. In general, a light level (i.e., growth on first quadrant) was observed with suspensions of greater than approximately 1000 cfu/mL, the moderate level (i.e., growth on first two quadrants) with suspensions greater than approximately 100,000 cfu/mL, and the heavy level (growth on three or all quadrants) with suspensions greater than approximately 10,000,000 cfu/mL.

A program of surveillance procedures for selected laboratory equipment also was used in the clinical laboratory. This included a time schedule (e.g., each time or use for pH meters, daily for incubators) and set tolerance limits for incubators, refrigerators, freezers, water baths, and pH meters.

Clinical Virology--

As described above, split samples coded as unknowns were also screened for viruses in parallel with routine clinical specimens. Unfortunately, no viruses were recovered from any of the 35 fecal samples received during April and May 1981. Therefore, the split-sample approach did not yield definitive data concerning laboratory precision for clinical virology.

A similar split-sample program was initiated in August 1981 in an effort to test the reproducibility of viral isolation in tube cultures from clinical specimens. Detailed results of this testing are presented in Table A-35 in Appendix A. Of the 33 participant samples used in this program, only two specimens yielded virus as part of the routine analysis while five isolates were recorded in QA testing. Notably, both samples found to be positive in routine testing were also positive in QA testing, although in one instance the isolation was made in different cell lines. These results also highlighted the low likelihood of recovering viruses from routine specimens when assay volumes were limited by tube culture inoculation. Subsequently, assay procedures were modified as described in Section 4E to increase the amount of sample inoculated into susceptible cell monolayers.

In addition, a specific quality assurance program was followed for viral identification. On a quarterly schedule, three ''unknown'' animal viruses (from laboratory stocks) were handled for identification using the serological protocols described under ''Laboratory Analysis--Clinical virology.'' An acceptable performance required the recovery of each unknown virus in at least one cell line and the correct identification of each isolate.

Electron Microscopy--

Photographs of each positive specimen were taken for documentation of visual identification. The electron micrographs were evaluated against micrographs published in peer reviewed journals with regard to size and distinctive morphological characteristics. Positive specimen material is maintained at -70°C for future reference. Poliovirus was used as the reference standard for size determination. All examinations were performed on the same JEOL 100CX electron microscope by the same microscopist. The microscope is maintained under a service contract and undergoes periodic maintenance and performance checks by qualified personnel.

In order to eliminate possible bias in the EM study, all stool specimens received from years 1980, 1981 and 1982 (the first year of irrigation) were coded and examined together. Some duplicates were included so that equal numbers of pre- and postirrigation specimens were examined. The identity of individual specimens remained unknown to the microscopist until all specimens had been examined.

The additional specimens received from the final year (1983) were examined separately, but included five coronavirus-positive and five negative specimens from the earlier examination for comparison. This examination was also performed under code.

Coronavirus-like particles were detected in only two of the five stools previously found positive for this agent. Subsequent examination of the three other specimens did reveal particles generally consistent with a coronavirus-like classification but with poorly defined fringe projections (perhaps deteriorated or antibody obscured). Such particles are difficult to detect during routine EM examination as fringed particles of all types are frequently encountered in stools. Additionally, all the coronavirus-like particles observed in the specimens to date have not had classical coronavirus morphology. These particles have an alternate or atypical appearance which is even more pleomorphic and more variably fringed than the classic propagated coronavirus. The occurrence of these particles has been widely reported, although their significance has not been established and it is not clear that such particles represent actual virus particles.

Environmental Samples--

In addition to the equipment and media performance testing described above for ''Clinical Bacteriology,'' the internal quality assurance program for analysis of environmental samples involved two approaches. Wastewater samples seeded with several laboratory strains of enteric bacteria were analyzed for the quantitative recovery of the unknowns. Likewise, selected known viruses were recovered and identified as described above for ''Clinical Virology.''

In addition, a series of split analyses for enterovirus concentration and assay on HeLa cells and for indicator bacteria enumeration by membrane filtration were incorporated into the enterovirus identification and/or routine wastewater analyses conducted monthly during April and May 1981. Results are summarized in Table A-36 in Appendix A. Both bacterial and viral analyses were within an acceptable repeatability range.

QA reprodubility data were generated by compiling data for indicator bacteria and total organic carbon (TOC) in Lubbock wastewater reported by the LCCIWR laboratory, the UTSA laboratory, and the UT Austin laboratory. Composite samples were collected by either SwRI or LCCIWR personnel, split and shipped as part of routine monitoring described previously. Results for total and fecal coliform bacteria recorded during baseline monitoring are presented in Table A-37 of Appendix A. Fecal coliform levels reported by LCCIWR and either UTSA or UTA laboratories during 1982 and 1983 are shown in Table A-38 in Appendix A. Similarly, split sample values for fecal streptococci are recorded in Table A-39. In most instances, total and fecal coliform and fecal streptococci values were well within the variability expected of a dilution-based bacterial assay. Indeed, when replicate results were reported by LCCIWR (see Tables A-38 and A-39), the duplicate value fell closer to that reported as the mean of triplicate platings by UT laboratories. Perhaps because of the larger number of samples compared, more interlaboratory discrepancies were observed with fecal coliform results.

To address these differences a series of in-house QA tests were conducted at UT Austin using samples collected on July 25 and 26, 1983 and August 8 and 9, 1983. Colonies counted as typical fecal coliforms (blue) and/or nonfecal coliforms (gray to cream-colored) were subcultured onto nonselective heart infusion agar. An oxidase test was completed on all isolates and selected bacteria were identified using the API 20E test system. Results of this QA testing are shown in Table A-40 in Appendix A. With the exception of three colonieswhose API profile was not definitive, all organisms recorded as fecal coliforms in samples collected on July 25 and 26, 1983 were identified as members of the Enterobacteriaceae family. Results for the Wilson wastewater sample collected on August 8 and 9 were less clear-cut. In this instance, as Aeromonas hydrophila. Perhaps more importantly, a significant number of nonfecal coliform colonies were oxidase negative. Of these, at least half were enteric bacteria. It should be noted that a total of 45 colonies were subcultured off of a single 47-mm diameter membrane filter and that some overlap of colonies may have occurred. Nonetheless, based on these observations, the value of 3.1×10^7 fecal coliform/100 mL reported for this Wilson sample (see Table P-3 in Appendix P) may be low. Aside from developmental work, little information identifying ''nonfecal'' coliforms appears in the published literature. Furthermore, these results are from a single sample which may or may not be representative of other assays or wastewater sources.

Overall, the agreement between laboratories for all indicator bacteria may be considered very good for microbial parameters. Furthermore, these comparative QA results show that the procedures used for sample shipment and analysis within 24-36 hours of collection resulted in valid experimental data with no remarkable sample deterioration. In addition, chemical analyses as demonstrated by TOC data shown in Table A-41 of Appendix A were quite comparable between laboratories.

Data Management

A sample identification system based on a coded label was used to preserve the integrity of the sample data. A computer-generated label was affixed to each sample's container (e.g., wastewater, aerosol, blood serum, fecal specimen, throat swab), each sample aliquot, and each source record (e.g., medical history, health diary). An alphanumeric code on the label specified the participant ID number, sample medium (e.g., blood, feces, wastewater), sampling period, type of sample analysis, etc., so the sample was uniquely identified. The key elements of the code were also printed in English on the label to facilitate sample processing. The sample code was reported to data management along with the analytical result and was keypunched and placed on the data base with the result. The sample code also functioned as the index key for the data base.

Data processing errors were minimized by judicious inspection and editing of participant-furnished data, inspection of field- and laboratory-reported data, key verification of keypunched data, and reliance on automated data processing accompanied by checks on the coherence of the data. Key processing steps were manually double-checked from file listings by the project manager to ensure they were performed correctly and completely. The values of key variables on the data base, such as the dependent variable in infection episodes, the aerosol exposure index and age, were visually inspected for reasonableness by the health watch manager and the project manager.

Archiving of Clinical Specimens

A portion of all clinical specimens (blood, feces, and throat swabs) taken in the health watch were preserved and frozen at -76°C. A cross-referenced catalog system allowed ready access to specific samples. Master lists of blood donors and clinical specimen donors were updated each period, reflecting each individual's cumulative participation in the health watch program. All illness and virus-positive fecal samples are archived at UTA.

Archived 1 mL aliquots of sera given by all participants during each blood collection in the entire study were transferred from UI and UTA to EPA-HERL upon completion of the laboratory phase of the LISS. Prior to shipment the inventory of archived aliquots was double-checked against a master listing of all blood samples obtained in the LISS. The archived sera have been stored between -35°C and -76°C at EPA-HERL.

J. STATISTICAL METHODS

Previous studies of the effect of wastewater and associated aerosols upon the health of such diverse groups as sewer and sewage treatment workers

(Clark et al., 1980; Sekla et al., 1980), agricultural workers (Shuval and Fattal, 1980), school children (Camann et al., 1980), and suburban residents (Johnson et al., 1980; Fannin et al., 1980; and Northrop et al., 1980) suggested that any health effects seen in the LISS were likely to be rather subtle. To ensure that the analysis of association of infection with exposure was sensitive enough to detect such effects, care was taken to employ statistical tests for which both the level and power could be calculated. In most instances this led to the use of rather simple tests of the main hypotheses. More elaborate and sophisticated analyses often involve tests whose power is unknown or known only approximately. These were considered to be exploratory techniques and were employed only after the primary test with controlled error probabilities had been conducted. Additional comprehensive and ad hoc analyses were performed to address the association of infection with exposure for data sets which were not amenable to the standard analysis.

The primary strategy was to divide the study participants into groups which received high or low exposure to the pathogens through aerosols, through direct contact with wastewater or by other means, and then to compare the incidence of infections in these two groups during a period of wastewater irrigation. Events which indicated an infection of an individual were either the occurrence of a seroconversion or a significant increase or detection of a fecal agent according to the definitions of infection events given in Section 4G. To permit use of sensitive statistical methods requiring that the dependent variable only assume the values 0 or 1, all multiple infection events were treated as single infection events in most statistical analyses performed; the exceptions are noted below. Thus, a value of 0 indicated the donor was not infected during the period of observation while 1 indicated the donor was newly infected. If exposure groups were comparable in every pertinent respect and the individuals' responses were independent, the proportion of infections occurring in the groups were compared in a simple contingency table analysis to determine if there was difference in incidence rates between the exposure groups. In cases where there was imbalance between the exposure groups with regard to important variables, it was necessary to stratify on these variables and compare rates within strata. Further, such variables were used as predictor variables in a logistic regression analysis to account for any differential effects they may have had on the infection rates.

Since individuals were clustered in households, the occurrence of their infections could have been correlated with those of other household members. The independence of infection events within households was evaluated as described below under Confirmatory Analysis.

The standard analysis may be viewed as consisting of these major stages:

1) Preliminary Analysis--comparison of the low exposure group (AEI<3) and the high exposure group (AEI$\geq$3) with respect to individual and household characteristics in order to determine if the two exposed groups differed significantly with regard to these factors.

2) Confirmatory Analysis--comparison of infection rates in the exposure groups to determine the presence of any association of infection and wastewater application. This was a major analysis of the study and resulted in a p-value for the rejection of each null hypothesis. The principal findings of the study will rest on the results of these analyses and their consistency with the other methods of inference employed.

3) Exploratory Analysis--investigation of whether the presence of infection was associated with a set of potential predictor variables and in particular with the degree of aerosol exposure.

A careful distinction between these stages was maintained during the analysis, discussion and conclusion sections of the study report. These stages of the standard analysis will now be described in more detail. The analysis of risk ratio scores, incidence density ratios, and various small data sets are presented later in this section.

Preliminary Analysis

Prior to conducting tests for association of infection rates and exposure, the exposure groups were compared with respect to other characteristics which could influence the outcome of the tests. The exposure groups therefore were compared by calculating the proportion in each category of each pertinent variable for each population to be tested (fecal donors and blood donors) in each of the six seasons of data and the baseline data set. For the baseline period comparison, the exposure groups were defined based on subsequent exposure during the first (spring 1982) irrigation season. A standard chi-square test for equality of proportions for 2xk contingency tables was used, where k is the number of categories of the characteristic and 2 is the number of exposure groups ($AEI<3$, $AEI\geq3$). A chi-square test may be used when fewer than 20% of the cells have an expected frequency of less than 5 and no cell has an expected frequency of less than 1 (Siegel, 1956). When these requirements were not met, adjacent categories of the characteristic were combined to increase the expected frequencies. For 2x2 contingency tables, a one-tailed Fisher's exact test was used whenever the expected frequency for any cell was less than 5. The range of the p-value, the number of observations in each exposure group, and the proportion (or percent) in each category of each exposure group was reported for each chi-square or Fisher's exact test.

From these tests, a judgment was made about the variable(s) to be used for stratification or included as explanatory variables. The relative importance, the consistency and magnitude of differences across seasons and the quality of the data for each variable was considered. To ensure consistency, a variable was considered for use as a stratifying variable if and only if 1) the variable was deemed to be epidemiologically important and 2) the hypothesis of equal proportions was rejected at the 0.01 level at least once or at the 0.05 level at least twice in the four irrigation seasons. If a variable met these criteria and if the number of observations was adequate, the variable was stratified prior to conducting the confirmatory analysis (discussed below). If not, the imbalance was reported and the confirmatory analysis was carried out without correction for that variable.

Confirmatory Analysis

Testing procedure--

Since individuals were clustered in households, the possible dependence of infections for individuals within households (i.e., intra-household correlation) was investigated. To determine the proper unit of analysis (household or individual), we examined whether, say, two individuals in the same household were more likely to both acquire infections than two individuals from different households. The approach was to fit a binomial distribution to the data. The binomial model was chosen because:

1) The data are binary.

2) If individuals are independent (i.e., no correlation within households) and the probability of infection is constant over individuals, the binomial is a plausible model.

3) Departures from the binomial can be examined by looking at the difference in observed and expected numbers of individuals. For example, in a household with two members donating specimens, the categories were:

    a) both members not infected
    b) one member infected
    c) both members infected

An excess in Category c indicated significant clustering of infections, i.e., if one member had the infection, the other was more likely to have the infection than was predicted by the binomial model. In summary, if the binomial model fitted (using a chi-square goodness of fit), there was no reason to suspect correlation.

In cases in which household clustering was not significant, a 2x2 contingency table analysis of infection status observed on individuals was used in a one-sided test of the hypothesis that the incidence rates of infection or seroconversion were the same for the high and low exposure groups. The investigation for each agent and observation period can be summarized by the following 2x2 contingency table

		Exposure	
		Low	High
Infected	Yes		
	No		
		n_1	n_2

wherein the column totals n_1 and n_2 are fixed. The two columns represent the outcomes of two binomial experiments, with probabilities of becoming infected being P_1 and P_2 in the low and high exposure groups, respectively. The statistic used for testing the null hypothesis $P_2=P_1$ against the alternative $P_2>P_1$ was

$$\chi^2 = \Sigma\,(\text{observed-expected})^2/\text{expected}$$

or when expected values were small, Fisher's exact test was used according to the rules stated above. The one-sided alternative was appropriate since $P_2<P_1$ suggests that people exposed to wastewater had fewer seroconversions than those not exposed, a seemingly remote and uninteresting possibility. Furthermore, the test of $P_2=P_1$ against $P_2>P_1$ was more powerful than the test against the two-sided alternative, given the same level and sample size. The range of the p-value, number of infections, and incidence rates in each exposure group were reported for each chi-square or Fisher's exact test.

Stratification--

When stratification was indicated in the preliminary analyses, the study groups were appropriately stratified and a simple contingency table analysis was conducted within each stratum. The results of the independent tests within strata were combined by the Mantel-Haenszel procedure (Kleinbaum et al., 1982). The range of the p-value, number of infections, and incidence rates in each stratum of each exposure group were reported for each Mantel-Haenszel test. Stratification was not performed unless the sample size criteria suggested by Mantel and Fleiss (1980) were met.

The preliminary and confirmatory analyses discussed above were performed using the BMDP4F (Dixon et al., 1983), SAS TFREQ (SAS, 1982), and Minitab (Ryan et al., 1982) statistical packages of computer programs.

Exploratory Analysis

The purpose of the exporatory analysis was to investigate whether the presence of infection was associated with a set of potential predictor variables. Primary interest was in determining if an association existed between the presence of infection and the degree of aerosol exposure. To achieve this goal, a stepwise logistic regression analysis was performed for each infection episode in which there was a higher rate of infection in the high exposure group ($AEI\geq3$) than in the low exposure group ($AEI<3$) and the high exposure level ($AEI>5$) than in the low ($AEI<1$) and intermediate ($1\leq AEI\leq5$) exposure levels. (The other infection episodes were not explored by this analysis because it was decided that they were unlikely to be associated with aerosol exposure.) The response variable was categorical in nature and equalled 0 if the individual was not infected and 1 if the individual experienced one or more infection events in the episode ''season.'' A set of descriptors for each individual was used as the predictor variables.

The analyses were performed using the BMDP-LR computer program (Dixon et al., 1983). LR is a stepwise logistic regression program designed to investigate the relationship between a binary response variable and a set of categorical and/or continuous predictor variables (Cox, 1970). It uses a maximum likelihood estimation approach for estimating the coefficients in the prediction equation and testing their significance.

The effects of each predictor variable used in the study were assessed through the usage of a maximum-likelihood-ratio chi-square test of the hypothesis that the explanatory power of that variable was zero. At each step of a given analysis a predictor variable was added to the constructed regression equation provided that it had the smallest chi-square p-value among all remaining predictor variables and that the p-value was less than 0.10. Similarly, a term could be removed from the equation at each step if it had the largest p-value among the predictor variables already entered into the equation and if the p-value exceeded 0.15. Occasionally, two or more predictor variables in an equation were so highly correlated that the regression analysis could not be run. In these cases, one or more of the collinear variables were deleted based on the magnitude of their correlation coefficient and the order in which they entered the prediction equation. This process was repeated for each response variable in every season.

The goodness of fit of the devised models in describing the relationship between the probability of infection and the selected predictor variables was assessed using a test developed by Hosmer and Lemeshow (1980); this actual test statistic is termed C^* in their article. The test is based on comparing the observed and expected frequencies of subjects having an infection. These subjects are grouped into ten cells based on their infection risk. The resultant test statistic has approximately a chi-square distribution. A small p-value (e.g., $p<0.10$) indicates that the prediction equation does not fit the data.

For each constructed model, approximate 90% confidence intervals were obtained for the odds ratio. This was calculated using an asymptotic normal approximation. Note that the logarithm of the odds ratio is the estimated coefficient of the predictor variable of interest. If the constructed confidence interval contained the value 1, it was concluded that the odds of having an infection were the same for the various categories of the predictor variable.

Analysis of Risk Ratio (RR) Scores

Risk ratio scores assigned in a symmetric manner to each independent infection episode were analyzed to provide a sensitive overview of any apparent association of infection events with wastewater aerosol exposure. Since it is based on all infection episodes observed in the LISS, the risk ratio score analysis provides an overview indication, which is both broad and sensitive, of any infection effects associated with wastewater spray irrigation. The risk ratio (RR) for exposure groups in an infection episode is the ratio of the infection incidence rate in the high exposure group divided by the infection incidence rate in the low exposure group.

If wastewater aerosol exposure were a major cause of infections in the study population, then a certain pattern should be evident in the infection incidence rates and risk ratios of the exposure groups and levels. The risk ratio for exposure groups should be large, perhaps RR≥3.0 for an episode with 5-8 newly infected donors or RR≥2.5 for an episode with 9 or more newly infected donors. The infection incidence rate should also be larger in the high exposure level than in the intermediate or low exposure levels, say by a factor of 2.0 or more both for high-to-intermediate levels and for high-to-low levels. If these group and level patterns both occurred in the same infection episode, this would be strong evidence for possible association of the infection events with wastewater aerosol exposure. Such an episode was assigned a risk ratio score of ++. The criteria are formally presented in Table 17. If a somewhat weaker pattern were apparent both in the risk ratio for exposure groups and in the incidence rates of the exposure levels, the infection episode was assigned a risk ratio score of +. The precise criteria for + are also given in Table 17.

Obviously some of the infection episodes assigned risk ratio scores of ++ or + will be due to chance. To control for this random effect, the same criteria were applied in a symmetric manner to the infection incidence rates of the low exposure group and level. Suppose that in an episode with 9 or more newly infected donors, the infection rate of the low exposure group exceeded the rate in the high exposure group by a factor of 2.5 or more and the infection rate in the low exposure level exceeded the rates in both the intermediate and high levels by more than a factor of 2. This episode was assigned a risk ratio score of – –, since the pattern was observed in the low exposure group and level rather than in the high exposure group and level. In this manner the risk ratio score criteria presented in Table 17 were developed. When no distinct pattern was evident in the group and level incidence rates (i.e., neither the criteria for a + score nor for a – score were met), the infection episode was assigned a risk ratio score of 0. Since smaller proportional incidence rates can be significant when the overall incidence rate becomes large, an alternate criterion involving the difference in the group incidence rates expressed as percentage points was developed for episodes with a large number of infected donors (see last column of Table 17).

It should be noted that the cutoff values in Table 17 defining a risk ratio score are arbitrary. If other cutoff values had been chosen, the scoring of risk ratios would have been different.

In summary, the risk ratio score criteria are symmetric with regard to the high and low exposure groups and levels (i.e., an infection pattern that would be scored + if the excess infections occurred in the high exposure group and level, would be scored – if the equivalent excess infections occurred in the low group and level). Thus, in the absence of any effect, random variation should produce an equal number of positive and negative risk ratio scores.

The risk ratio score criteria presented in Table 17 were applied to every infection episode. The sign test can be used to determine whether a preponderance of positive risk ratio scores is statistically significant,

TABLE 17. RISK RATIO SCORE CRITERIA

Risk ratio score		Criteria for exposure group and level infection rates (GIR_g and LIR_1)[a] by number infected in episode (both group and level criteria must be satisified)		
		3-4	5-8	9 or more
++	2 groups:	$GIR_{Lo}=0$	$GIR_{Hi}/GIR_{Lo}>3$	$GIR_{Hi}/GIR_{Lo}>2.5$ (or $GIR_{Hi}-GIR_{Lo}>15\%$ points)
		and	and	and
	3 levels:	$LIR_{Hi}/LIR_{Int}>3$	$LIR_{Hi}/LIR_{Int}>2$ and $LIR_{Int}/LIR_{Lo}\geq 1$	$LIR_{Hi}/LIR_{Int}>2$ and $LIR_{Hi}/LIR_{Lo}>2$
+	2 groups:	$GIR_{Hi}/GIR_{Lo}>2.5$	$GIR_{Hi}/GIR_{Lo}>2$	$GIR_{Hi}/GIR_{Lo}>1.5$ (or $GIR_{Hi}-GIR_{Lo}>10\%$ points)
		and	and	and
	3 levels:	$LIR_{Hi}/LIR_{Int}>2$ and $LIR_{Hi}/LIR_{Lo}>2$	$LIR_{Hi}/LIR_{Int}>1$ and $LIR_{Hi}/LIR_{Lo}>1$	$LIR_{Hi}/LIR_{Int}>1$ and $LIR_{Hi}/LIR_{Lo}>1$
0		No distinct pattern	No distinct pattern	No distinct pattern
-	2 groups:	$GIR_{Lo}/GIR_{Hi}>2.5$	$GIR_{Lo}/GIR_{Hi}>2$	$GIR_{Lo}/GIR_{Hi}>1.5$ (or $GIR_{Lo}-GIR_{Hi}>10\%$ points)
		and	and	and
	3 levels:	$LIR_{Lo}/LIR_{Int}>2$ and $LIR_{Lo}/LIR_{Hi}>2$	$LIR_{Lo}/LIR_{Int}>1$ and $LIR_{Lo}/LIR_{Hi}>1$	$LIR_{Lo}/LIR_{Int}>1$ and $LIR_{Lo}/LIR_{Hi}>1$
--	2 groups:	$GIR_{Hi}=0$	$GIR_{Lo}/GIR_{Hi}>3$	$GIR_{Lo}/GIR_{Hi}>2.5$ (or $GIR_{Lo}-GIR_{Hi}>15\%$ points)
		and	and	and
	3 levels:	$LIR_{Lo}/LIR_{Int}>3$	$LIR_{Int}/LIR_{Hi}>2$ and $LIR_{Lo}/LIR_{Int}\geq 1$	$LIR_{Lo}/LIR_{Int}>2$ and $LIR_{Lo}/LIR_{Hi}>2$

[a] GIR_g - infection incidence rate of AEI group g, %
LIR_1 - infection incidence rate of AEI level 1, %
GIR_{Hi}/GIR_{Lo}=RR for exposure groups
LIR_{Hi}/LIR_{Lo}=RR for exposure levels

provided the observations (i.e., infection episodes) are jointly independent. Six interpretable groups of jointly independent and mutually exclusive infection episodes were defined, based on the criteria given in Table 15. A one-sided sign test of the number of positive scores (++ or +) compared to the number of negative scores (- - or -) was conducted for each jointly independent group to determine if there was a significant excess of positive risk ratio scores for the infection episodes in the group.

Groups C and F of control infection episodes should have symmetric frequency distributions of RR scores about the score of 0. Under the null hypothesis of no association between infections and wastewater aerosol exposure, Groups A, B, D and E of exposure infection episodes should also exhibit frequency distributions of RR scores which are symmetric about 0, with no excess of positive scores over negative scores. If there were a significant excess of positive scores (at $\alpha=0.05$ by the one-sided sign test) in a group of exposure infection episodes, this would provide an overall indication of apparent association of infections with wastewater aerosol exposure.

Analysis of Incidence Density Ratios (IDR) using Test-based Confidence Intervals

Test-based confidence intervals were constructed to determine if the ratio of the incidence density (ID) of highly exposed participants to the incidence density of less exposed participants significantly exceeded one. This incidence density ratio (IDR) analysis was applied both to new infections detected serologically and to new self-reported acute illnesses.

The average rates of infection events determined as seroconversions (i.e., fourfold or greater increases in titer in paired sera) were estimated as incidence densities for the low ($AEI<1$), intermediate ($1\leq AEI\leq 5$), and high ($AEI>5$) exposure levels and for the low ($AEI<3$) and high ($AEI\geq 3$) exposure groups. The infection ID was expressed as the number of new infections per hundred person-years of observation:

$$ID = \frac{\text{No. of New Infections in Time Interval}}{\text{No. of Person-days Observed During Interval}} \times (365.25 \text{ days/yr}) \times (100 \text{ years})$$

ID was calculated for the seven time intervals defined as serologic periods of observation in Section 4G and the ''irrigation'' interval from January 1982 to October 1983 spanning all observed periods of irrigation.

In accumulating the numerator and denominator of ID, the participant's aerosol exposure index for the period of interest was used to categorize that participant by exposure group or exposure level. For example, if a person had an infection in the spring of 1982 and was considered to be in the high exposure level during irrigation in spring 1982 and in the intermediate exposure level during irrigation for the entire year of 1982, that person's infection and cumulative person-days of observation would be included in the high exposure level in the ID calculation for the spring of 1982, but in the intermediate exposure level in the ID calculation for the 1982 time interval.

The exception to this rule was in the ID calculation for the entire irrigation time interval. In this case, when an individual's exposure level changed between irrigation periods, his infection events and person-days of observation were accumulated in the proper exposure level for each irrigation period. For example, suppose a person had two infection events to a group of four agents while in the high exposure level in spring 1982, one infection while in the intermediate exposure level during summer 1982, and no infections while in the high exposure level in either spring or summer 1983. Suppose the person had 600 agent-person-days of observation each in spring 1982, summer 1982, and spring 1983, but 400 agent-person-days of observation in summer 1983. Then, in the ID calculation for the high exposure level, the person would contribute 2+0+0=2 infection events and 600+600+400=1600 person-days of observation. He would also increase the numerator and denominator of ID for the intermediate exposure level by 1 infection event and 600 person-days, based on his summer 1982 experience. As this example illustrates, a person's experience could be allocated to several exposure levels or groups in the ID calculation for the irrigation time interval. In cases where a seroconversion could not be located to a specific irrigation season, the aerosol index for the appropriate year (1982 or 1983) was used to categorize the participant's exposure as high, intermediate or low. The infection and the appropriate high person-days of observation were then accumulated in that exposure level for the entire year.

Three incidence density ratios (IDRs) were calculated. For exposure groups, IDR = ID_{Hi}/ID_{Lo}. For exposure levels, two IDRs were calculated: for the high-to-intermediate exposure levels (ID_{Hi}/ID_{Int}) and for the high-to-low exposure levels (ID_{Hi}/ID_{Lo}). We are interested in testing the null hypothesis of no association between infections and wastewater irrigation against the alternative of a positive association. This is equivalent to determining if IDR is significantly larger than 1.0.

Confidence intervals were constructed for each IDR using Miettinen's test-based confidence interval approach as described by Kleinbaum et al. (1982) on pages 300-302. They point out that the statistical properties of the test-based confidence interval need additional study. Test-based intervals tend, on the average, to be a little narrower than Taylor series intervals, but the discrepancy is usually negligible when $0.25 \leq IDR \leq 4$.

To make clear the assumption of test-based confidence intervals, these assumptions are stated as they apply to IDR = ID_{Hi}/ID_{Lo} for the two exposure groups. It is assumed that the allocation of the total number n of infection events observed in both exposure groups into the high group and into the low group is a binomial experiment. The probability p_2 that a given infection will occur in the high exposure group is estimated as the proportion of the total person-years of observation that were observed for the high group [i.e., $PT_2/(PT_1+PT_2)$]. Each infection is assumed to have the same probability p_2 of occurring in the high exposure group. The occurrence of consecutive infections are also assumed to be independent with respect to the exposure group in which they occur. Finally, since the normal distribution approximation to the binomial distribution is used, the expected number of infections in both exposure groups should be large enough, say $np_1 \geq 5$ and $np_2 \geq 5$.

Infection IDRs and their 90% and 95% confidence intervals were calculated for each individual serologic agent and for the six groupings of serologic agents given in Table 18. Results from the entire baseline and entire irrigation periods were compared, both by exposure levels and by exposure groups. Results for individual agents and the six agent groupings were also calculated for each of the eight time intervals by exposure levels. Whenever the 90% or 95% test-based confidence interval for IDR did not include 1.0, this result was reported, provided the expected number of infections in each of the exposure levels or groups compared was at least 2.0. An IDR was considered to be significant if its 95% confidence interval did not include 1.0 and if $np_1 \geq 2$ and $np_2 \geq 2$.

TABLE 18. DEFINITIONS FOR AGENT GROUPINGS IN SEROLOGIC DATA ANALYSIS

SNV	All nonpolio viruses tested by serum neutralization. This group includes all coxsackieviruses, echoviruses, and adenoviruses.
WWV	All viruses recovered from Lubbock wastewater during the period of observation (see Tables 25-27 and 39). This group is a large subset of the SNV grouping, consisting of coxsackieviruses and echoviruses (see Table 99).
POR	Serum neutralization (SNV) viruses which caused too few infections during the period of observation to constitute a distinct infection episode. Since wastewater contains many infectious agents, it was felt that ''sporadic infections'' by a variety of agents might be the most subtle effect of wastewater exposure.
ADEN	Adenoviruses 3,5, and 7. (This grouping was used only for calculating incidence densities).
COXB	Coxsackieviruses B2, B4, and B5. (This grouping was used only for calculating incidence densities).
ECHO	Echoviruses 1, 3, 5, 9, 11, 17, 19, 20, 24. (This grouping was used only for calculating incidence densities).

The average rates of self-reported acute illness were also estimated as incidence densities for the three exposure levels and two exposure groups. The illness ID was expressed as the number of new illnesses per 1000 person-days of observation. The ID was determined for total acute illnesses and for the subcategories of respiratory illness, gastrointestinal illness, and other acute illnesses such as eye and ear infections and skin conditions. ID was calculated for time intervals of ''months'' which were usually of 4-weeks duration. Otherwise, the illness ID was calculated in the same manner as the seroconversion ID. Illness IDRs and their test-based confidence intervals were also computed for exposure groups and for exposure levels in the same manner as the seroconversion IDRs.

The assumption in using a test-based confidence interval of a binomial experiment regarding the allocation of events among the two exposure groups or levels in the IDR appears reasonably valid, both for serologically-detected infections and for self-reported acute illnesses. As in most LISS analyses, the assumption of independence may not be strictly valid because of the greater likelihood of within-household transmission of the infectious agent. However, over the 6-month or greater time interval of the seroconversion ID and the 4-week time interval of the illness ID, this effect is likely to balance out over the two groups being compared. There are intrinsic differences among individuals which cause them to respond differently, regarding the probability of both a seroconversion and of self-reported illness, to a given challenge by the same agent. The serological overreactors and underreactors are likely to be evenly distributed throughout the study population, and hence balanced among exposure levels and groups. Because self-reporting of acute illness could be biased by the odor of nearby wastewater irrigation, illness overreactors and underreactors might not be distributed in a balanced manner by exposure levels and groups.

By using a population-time denominator for ID, the IDR analysis using a test-based confidence interval takes proper account of periods of non-observation or nonrisk (i.e., missing reporting periods or days spent outside the study area). When applied to groups of serologic agents, multiple sites of acute illness and/or consecutive periods of observation, this analysis takes proper account of the multiple infection or illness events which a participant is liable to experience. On the other hand, the IDR analysis is not valid unless a large number of infection or illness events occur. Thus, the IDR analysis has most value to the LISS in providing an overview interpretation of observed gradients in incidence density by exposure level or exposure group when the infections to groups of serologic agents are observed over a long time interval (entire baseline or entire irrigation period) or when total acute illness is observed.

Other Analyses of Apparent Association of Infections with Exposure

For small sets of data, other analyses were performed as appropriate to investigate the apparent association of the occurrence, prevalence or incidence of infections with wastewater aerosol exposure and other pertinent factors. Since incidence data was usually lacking and the data sets were often small, definitive evidence of association was difficult to establish through these analyses.

A descriptive analysis was conducted to determine the time period(s) with the highest rates of occurrence, prevalence or incidence. Unless there was a higher rate of occurrence during one or more periods of irrigation, it was decided that there was no apparent association with wastewater exposure.

When a high rate of occurrence was observed during an irrigation period, the mean AEI of the infected donors was compared to the mean AEI of the noninfected donors from the same irrigation period. If the mean AEI of the infected donors was greater, a one-sided t test of the difference in the mean aerosol exposure of the populations of infected donors and noninfected donors was performed. A natural logarithm transformation of AEI was always necessary to equalize the variances, as determined by the F-test, or to minimize the variance inequality. The geometric means and the degree of apparent association indicated by the p-value of the one-sided t test were reported.

The possible associations of the cluster of occurrences with other plausible environmental factors were also investigated as alternative explanations. These factors included patronage of local restaurants, use of an evaporative cooler for home air conditioning, and contamination of the drinking water wells of rural households. Since each environmental factor was categorical, the 2x2 contingency table of infection status and environmental exposure was analyzed for association by a one-sided Fisher's exact test. The p-value was reported to indicate the degree of apparent association.

K. INTERPRETATION OF STATISTICAL RESULTS

The LISS employed four methods of inference to investigate the possible association of infections with wastewater aerosol exposure in the specific episodes of infection which were observed in the study population. These inferential methods are: 1) risk ratio (RR) scoring, 2) test-based confidence intervals of the incidence density ratio (IDR) of high-to-intermediate and high-to-low exposure levels for serologic infection episodes, 3) confirmatory statistical analysis (CA), and 4) exploratory logistic regression (ELR) statistical analysis.

A score was assigned by each of the four methods of inference to every infection episode. The RR score was assigned by the criteria previously given in Table 17. The score for the IDR method was based on the significance of the IDR confidence interval (CI), provided the expected number of infection events in each of the exposure levels compared was at least 2.0:

IDR score = - if $IDR \leq 1.0$
0 if $IDR > 1.0$, but 90% CI includes 1.0
+ if 90% CI does not include 1.0
++ if 95% CI does not include 1.0

IDR scores were assigned to the IDRs both for the high-to-intermediate exposure levels and for the high-to-low exposure levels. The score for the confirmatory analysis method was based on the p-value for the one-tailed Fisher's exact test:

CA score = $- -$ if $p \geq 0.95$
$-$ if $0.95 > p > 0.15$
0 if $0.10 < p \leq 0.15$
$+$ if $0.05 < p \leq 0.10$
$++$ if $0.01 < p \leq 0.05$
$+++$ if $p \leq 0.01$

The score for the exploratory logistic regression method was based on the p-value of chi-square to enter or to remove for the AEI predictor variable at the last step of the logistic regression model selection:

ELR score = $(-)$ if exploratory analysis not performed because group or level $RR \leq 1.0$
$-$ if $p > 0.25$
0 if $0.10 < p \leq 0.25$
$+$ if $0.05 < p \leq 0.10$
$++$ if $0.01 < p \leq 0.05$
$+++$ if $p \leq 0.01$

Two summary tables of the scores from all four of these inferential methods were presented: one for every control infection episode and another for every exposure infection episode. It is expected that a number of the statistically significant associations found by some methods employed in certain infection episodes will not be supported by the results from the other inferential methods. The four inferential methods complement each other to provide a balanced assessment of the association of infection events with wastewater aerosol exposure in a specific infection episode. Since each method also has its deficiencies, all four methods are needed to achieve a proper interpretation about the strength of the association.

It is important to identify the infection episodes for which there is strong and consistent evidence of association among the inferential methods. These infection episodes warrant additional scrutiny.

Strength of the association of infections and exposure in an infection episode was determined based on the most statistically significant result from the CA, ELR and IDR methods. Consistency in support of the association among the other inferential methods (CA, ELR, IDR and RR score) was also required. Presented in Table 19 are the precise criteria which were employed to classify the strength and consistency of the evidence of association in an infection episode as ''good'' or ''marginal'' based on the four inferential methods.

The LISS obtained additional pertinent information which was not employed in the inferential methods used to compile the list of infection episodes with good or marginal evidence of association. Enteroviruses recovered from regular wastewater samples were identified. Thus, whether the specific agent(s) of the infection episode were recovered from the wastewater during the irrigation period can be ascertained. This wastewater evidence is of better quality for some monitored agents than for others as shown in Table 20. A relative aerosol exposure measure (RAEM) was calculated for each microorganism group monitored in the aerosol sampling. Comparison

TABLE 19. CRITERIA FOR STRENGTH AND CONSISTENCY OF APPARENT ASSOCIATION OF INFECTIONS WITH WASTEWATER AEROSOL EXPOSURE IN INFECTION EPISODES

Classification	Criteria
Good	1. Strength of statistically significant association by at least one of the three methods employed:
	a. confirmatory analysis (CA): $p \leq 0.05$ (score $\geq$ ++)
	b. exploratory logistic regression (ELR): $p \leq 0.05$ (score $\geq$ ++)
	c. Incidence density ratio (IDR) of exposure levels: 95% CI does not include 1.0, both for Hi/Int and Hi/Lo (++ and ++)
	and 2. Consistency in support for association, either
	a. by another method at the degree of strength in 1 above
	or b. by at least three methods at lesser strength:
	(1) CA: $p \leq 0.10$ (score $\geq$ +) (or $p \leq 0.15$ if RR score = ++)
	(2) ELR: $p \leq 0.10$ (score $\geq$ +) (or $p \leq 0.15$ if RR score = ++)
	(3) IDR either both 90% CIs do not include 1.0 (+ and +) or one 95% CI does not include 1.0 (++ and 0 or 0 and ++)
	(4) risk ratio (RR) score = + or ++
Marginal	1. Strength of the association approaches statistical significance by at least one of the three methods employed:
	a. CA: $p \leq 0.10$ (score $\geq$ +)
	b. ELR: $p \leq 0.10$ (score $\geq$ +)
	c. IDR either both 90% CIs do not include 1.0 (+ and +) or one 95% CI does not include 1.0 (++ and 0 or 0 and ++)
	and 2. Consistency in support for possible association, either
	a. by another method at the degree of strength in 1 above
	or b. by at least three methods at lesser strength:
	(1) CA: $p \leq 0.15$ (score $\geq$ 0)
	(2) ELR: $p \leq 0.15$ (or $p \leq 0.20$ if RR score = ++)
	(3) IDR: one 90% CI does not include 1.0 (+ and 0 or 0 and +)
	(4) RR score = + or ++

TABLE 20. CRITERIA FOR JUDGING QUALITY OF WASTEWATER EVIDENCE FOR EACH MICROORGANISM

Category of quality	Quality of evidence of agent in sprayed wastewater			
	1. Excellent	2. Good	3. Fair	4. Presumptive
Source measurement (Hancock wastewater)				
Frequency	Frequent[a]	Frequent	Occasional[b]	None
Specificity	Serotype	Species/genus	Genus/species	
Transmission measurement (Wilson wastewater[c])				
Frequency	Frequent	Frequent	None	None
Specificity	Serotype	Species/genus		
Agent monitored in clinical specimens (suitable as a dependent variable in the statistical analysis)	Specific coxsackievirus[d] Specific echovirus[d]	Salmonella[e] Shigella[e] Yersinia enterocolitica[e] Campylobacter fetus[e] Fluorescent Pseudomonas[e] Klebsiella[e] Mycobacteria (atypical)[g] Candida albicans[e]	Legionella pneumophila[f] Staphylococcus aureus[e] Proteus/Citrobacter[e] Aeromonas/Serratia[e]	Hepatitis A virus[f] Adenovirus[f] Reovirus[f] Rotavirus[f] Norwalk virus[f] Virus-like particles[h]

[a] Frequent: at least one measurement every four weeks.
[b] Occasional: about one measurement per irrigation season.
[c] Feces of rural donors may be substituted when the dependent variable is serologic.
[d] Infections determined from serologic or fecal isolate data.
[e] Infections determined from fecal isolate/level data.
[f] Infections determined from serologic data.
[g] Infections determined from skin test data.
[h] Infections determined by electron microscopy of fecal specimens.

of the period of occurrence of the infection episode to the RAEM rank of the agent's microorganism group in that season can determine whether the episode occurred in the season of highest exposure to the agent via wastewater aerosols. Alternative sources of exposure were also investigated. Contaminated drinking water was evaluated for the subset of under 20 households whose drinking water wells were being monitored at the time of the infection episode.

A retrospective survey of routine fecal and requested throat swab donors was conducted to determine the frequency with which they had eaten at each of the restaurants in Wilson. A special ELR analysis (Analysis 2) was performed to evaluate the restaurant etiology as an alternative explanation to wastewater aerosol exposure. Eating at the restaurants was evaluated both as an alternative and as an additional explanation. Another ELR analysis (Analysis 3) was performed to investigate alternative explanations besides the restaurants. AEI was excluded from the eligible predictor variables for infection episodes in which it had been significant to determine if another variable would enter the model in its place.

A summary table of the evidence from the additional data sources described above will be prepared for each of the infection episodes with good or marginal evidence of wastewater aerosol exposure association. A review of this evidence regarding an apparently associated episode may discredit the association by identifying a more plausible alternative explanation. Any episodes surviving this winnowing process are more likely to be causally related to wastewater aerosol exposure.

Finally, the separate findings from each observed episode of infection will be considered together to draw conclusions regarding wastewater aerosol exposure and the incidence of infection. The relative quality and reliability of the data upon which each finding was based will be utilized to rank the findings. Consistency in the pattern of evidence across several infection episodes would probably be needed to indicate a relationship between infections and wastewater irrigation.

SECTION 5

RESULTS

A. MICROORGANISM LEVELS IN WASTEWATER

24-Hour Composite Samples--Overview

Environmental monitoring spanned a 4-year period beginning in June 1980 prior to on-site irrigation and continuing through September 1983. During the 2 baseline years of the LISS, composite wastewater samples were collected at both the Lubbock Southeast Trickling Filter Plant and the Wilson Imhoff tank. Beginning with the delivery of treated Lubbock sewage to the Hancock farm site in February 1982, pipeline effluent and reservoir water were analyzed for a variety of microbiological and selected physical-chemical parameters. Results of these analyses for each sample are presented in Tables P-1, P-2 and P-3 in Appendix P for Lubbock wastewater (and subsequently pipeline effluent), Hancock farm reservoir water and Wilson wastewater, respectively. To allow a comparative overview of these analytical parameters, geometric mean values for each of the seasonal irrigation periods also have been calculated and are summarized in Table 21.

From its sample profile, Lubbock wastewater effluent may be classified as relatively strong based on both microbial and chemical analyses. A review of data presented in Table P-1 in Appendix P shows that fecal coliform levels in effluent sampled at the Lubbock treatment plant routinely exceeded 10^4 cfu/mL, while total organic carbon (TOC) values ranged from 40 mg/L to over 200 mg/L. During the first 2 years of the study, total enterovirus levels as measured on HeLa cell monolayers ranged from 0.045 pfu/mL to over 1.0 pfu/mL in the summer of 1980.

The first pipeline effluent was sampled at the Hancock farm in February 1982 and represented a highly atypical sample microbiologically. Once a daily wastewater flow to the Hancock site was established, the initial microbial and physical profile of the wastewater delivered to the irrigation site was not dissimilar from the wastewater previously characterized at the treatment plant (see Table P-1). However, the quality of the pipeline effluent as indicated by TOC and TSS improved considerably after the first irrigation period (see Table 21). In 24-hour composite samples, maximal viral levels of about 0.1 pfu/mL were observed during spring monitoring, while levels approaching 0.5 pfu/mL were detected during the summer 1982 irrigation season. A similar pattern of enteric viruses enumerated on HeLa cell monolayers was observed during 1983. While viral levels in pipeline effluent did not reach the highest levels seen in June 1982, the number of viruses recovered remained relatively constant from late June through August 1983 at over 0.25 pfu/mL.

TABLE 21. QUALITY OF WASTEWATER APPLIED BY SPRINKLER IRRIGATION

Measurement by irrigation period	Wastewater source: Pipeline effluent[a]	Wastewater source: Reservoir effluent[b]
Total Organic Carbon (mg/L)		
Feb-Apr 1982	105	--
Jul-Sep 1982	61	22
Feb-Apr 1983	67	26
Jul-Sep 1983	30	25
Total Suspended Solids (mg/L)		
Feb-Apr 1982	149	--
Jul-Sep 1982	78	27
Feb-Apr 1983	72	29
Jul-Sep 1983	26	27
Fecal Coliforms (colony forming units/mL)		
Feb-Apr 1982	43,000	--
Jul-Sep 1982	13,000	130
Feb-Apr 1983	20,000	52
Jul-Sep 1983	9,000	29
Enteroviruses (plaque forming units/mL)		
Feb-Apr 1982	0.04	--
Jul-Sep 1982	0.05	0.003
Feb-Apr 1983	0.07	0.002
Jul-Sep 1983	0.17	0.001

[a] Geometric mean of four to eight 24-hour composite samples.

[b] Geometric mean of four or five grab samples.

Similar data for Hancock reservoir water collected beginning in June 1982 are shown in Table P-2 in Appendix P and summarized in Table 21. A comparison of both indicator bacteria and virus levels shows that, in general, organism concentrations in reservoir water were two to three orders of magnitude lower than comparable pipeline effluent. Of the 19 samples of reservoir water concentrate which were assayed in two cell lines, enteroviruses were detected in only 12 samples with a maximal level of about 0.06 pfu/mL. In most of the reservoir samples viral levels were at or below the detection sensitivity of the recovery procedures employed.

Microorganism concentrations in Wilson wastewater are profiled in Table P-3 in Appendix P. Primarily as a result of the smaller collection system in the city of Wilson, greater variability in organism levels was observed in wastewater samples. Fecal coliform densities ranged from 10^3 to in excess of 10^5 cfu/mL during the monitoring period. Similarly, total enteric virus levels as assayed on HeLa cell monolayers varied from no virus detected in five samples to over 3 pfu/mL in two samples.

Of particular interest in the 1982 monitoring period were the unusually high levels of polioviruses persisting in the Wilson wastewater from March to May. Viruses did not appear in the Wilson sewage until 3 weeks after the first Lubbock wastewater (also containing predominantly polioviruses) was collected at the Hancock farm. Notably, polioviruses 2 and 3 comprised most of the identified isolates from both sources. Although less dramatic, a similar pattern of poliovirus prevalence was observed in Wilson wastewater from March to May during 1983.

Extensive bacterial screens were completed on selected 24-hour composite samples in an effort to better define the microbial content of wastewater destined for spray irrigation. Identical analyses were completed on Wilson sewage to determine if any unique microbial differences existed between the Lubbock and Wilson wastewaters. Results are presented in Tables 22, 23 and P-4 (Appendix P) for Lubbock wastewater, Hancock reservoir water, and Wilson wastewater, respectively. The most prevalent *Enterobacteriaceae* species encountered in wastewater from either Lubbock or Wilson included *Citrobacter*, *Enterobacter*, *Escherichia* and *Klebsiella*. *Aeromonas hydrophila* was the most abundant non-*Enterobacteriaceae* member recovered followed by *Pseudomonas* species. In fact, *Aeromonas hydrophila* was the most prevalent organism detected in wastewater. No unexpected differences were observed in microbial profiles. The effectiveness of ponding for the reduction of microbial numbers was evident both by the lower levels and the reduced diversity of organisms seen in a single bacterial screen completed on a sample from the Hancock reservoir (see Table 23).

24-Hour Composite Samples--Bacterial Pathogens

Specific attempts were made during this study to isolate major enteric bacterial pathogens from wastewater, including *Salmonella* sp., *Shigella* sp., *Campylobacter jejuni*, *Yersinia enterocolitica*, and *Legionella pneumophila*. In both Lubbock and Wilson wastewaters, *Salmonella* sp. were recovered most frequently with isolations from 62% and 35% of the samples tested, respectively. *Campylobacter jejuni* and *Yersinia enterocolitica* were recovered

TABLE 22. BACTERIAL SCREENS[a]—LUBBOCK, TEXAS

	Sampling date							
	1980			1981		1982		
Organisms (10^3 cfu/mL)	Jun 3-4	Jul 28-29	Nov 3-4	Apr 20-21	Jul 20-21	Feb[b,c] 15-16	Mar 22-23	Jul 26-27
Enterobacteriaceae								
Citrobacter amalonaticus	-	-	-	-	-	0.05	-	-
Citrobacter diversus	-	-	-	-	-	0.05	-	-
Citrobacter freundii	15	-	10	-	5	0.43	4	6.6
Enterobacter aerogenes	5	10	10	-	-	-	-	3
Enterobacter agglomerans	16	10	20	10	-	0.13	-	10
Enterobacter cloacae	20	30	-	20	15	-	2	16
Enterobacter sakazakii	5	-	-	-	-	-	-	-
Escherichia coli	20	20	30	20	25	0.4	4	10
Escherichia coli alkalescens	-	-	-	50	-	-	-	-
Klebsiella oxytoca	7	-	10	20	5	-	4	3
Klebsiella ozaenae	5	-	-	-	-	0.025	-	-
Klebsiella pneumoniae	5	-	10	-	15	0.025	2	6.6
Morganella morganii	-	-	-	-	-	-	1	-
Providencia alcalifaciens	-	-	-	10	-	-	-	3
Providencia rettgeri	-	-	-	-	5	-	-	-
Serratia liquefaciens	-	-	-	-	-	-	-	3
Serratia marcescens	5	-	-	-	-	-	-	-
Serratia rubidaea	3	-	-	10	-	-	-	-
Vibrio fluvialis	-	-	-	-	-	-	-	56
Yersinia enterocolitica	10	-	-	-	-	-	1	-
Yersinia kristensenii	-	-	-	-	-	0.025	-	-
Non-Enterobacteriaceae								
Achromobacter spp.	-	-	-	10	-	-	3	-
Achromobacter xylosoxidans	5	20	-	-	-	-	5	10
Acinetobacter calcoaceticus var. Lwoffi	-	-	-	-	-	-	-	3
Aeromonas hydrophila	93	560	590	510	210	1.3	8	150
Alcaligenes sp.	5	10	-	-	-	-	1	10
API—Group I[d]	-	-	-	-	10	-	1	3
CDC Group II K-2	-	-	-	10	-	-	-	-
CDC Group V E-2	-	-	-	-	-	-	-	10
Chromobacterium sp.	5	-	-	-	-	-	-	6.6
Eikenella corrodens	-	-	10	-	-	-	-	-
Flavobacterium odoratum	3	-	-	-	-	-	1	-
Fluorescent Pseudomonas gp.	-	-	-	-	5	-	-	-
Pasteurella multocida	10	-	-	-	-	0.13	-	-
Pseudomonas cepacia	10	10	-	10	10	0.025	-	20
Pseudomonas fluorescens	15	10	-	10	-	-	1	-
Pseudomonas maltophilia	5	-	-	-	-	-	-	-
Pseudomonas putida	30	-	30	20	10	-	3	-
Pseudomonas putrefaciens	60	20	10	20	-	-	-	-
Pseudomonas stutzeri	10	-	-	-	-	-	-	-
Pseudomonas sp., other	25	140	-	-	25	0.025	8	-
Vibrio alginolyticus	-	-	-	-	5	-	-	-

a Highest levels observed on either MacConkey agar or brilliant green agar and identified by API 20E biochemical tests.

b On February 15, 1982 the sample source was changed from the trickling filter to the pipeline.

c Chlorination of wastewater effluent at treatment plant.

d A group of organisms which to date have been described by CDC and have been designated temporarily by API as API Group I.

TABLE 23. BACTERIAL SCREEN[a]--HANCOCK RESERVOIR

Organisms (10^3 cfu/mL)	Sampling date Jul 26-27, 1982
Enterobacteriaceae	
Enterobacter cloacae	0.4
Klebsiella oxytoca	0.1
Klebsiella ozaenae	0.1
Non-Enterobacteriaceae	
Achromobacter xylosoxidans	0.9
Acinetobacter calcoaceticus var. Lwoffi	0.2
Aeromonas hydrophila	4.3
Alcaligenes sp.	0.5
CDC Group V E-2	0.1
Pseudomonas sp.	0.5
Pseudomonas cepacia	0.1
Pseudomonas maltophilia	0.3

[a] Highest levels observed on either MacConkey agar or brilliant green agar and identified by API 20E biochemical tests.

in approximately one-third of the Lubbock samples tested for these organisms while only Yersinia was detected in a single bacterial screen of Wilson effluent. Shigella sp. were detected in Lubbock wastewater in 12% of the samples analyzed. The only major enteric pathogen recovered from reservoir water was a single isolation of Salmonella.

Table 24 summarizes the results of UI efforts to isolate Legionella from wastewater samples. No isolates of these agents were recovered from any of the seven samples processed, although antigens from a variety of serogroups and species were repeatedly demonstrated in wastewater samples and in tissues of guinea pigs inoculated with those samples. Most isolates of potential Legionella group agents grew readily on TSA or blood agar. One isolate, not growing on TSA in the UI laboratory, was forwarded to the Illinois Department of Public Health Bacteriology Laboratory where it grew on a number of media, including TSA, suggesting the isolate was not Legionella.

The UI experience in isolation attempts of Legionella from water samples is not unusual; others have also been unable to recover viable Legionellae from DFA positive samples. Factors influencing the inability to recover Legionella include the susceptibility of experimental animals to Legionella infection, viability and virulence of Legionella present in wastewater samples, and the levels of both Legionella-group and non-Legionella agents present in those samples.

TABLE 24. SPECIES OF LEGIONELLA DETECTED[a] IN WASTEWATER SAMPLES BY DIRECT FLUORESCENT ANTIBODY STAINING OF THE ORIGINAL SAMPLES OR TISSUES FROM GUINEA PIGS INOCULATED WITH THOSE SAMPLES

	L. pneumophila						L.	L.	L.	L.	L. longbeacheae	
Sample	1	2	3	4	5	6	bozemannii	dumoffii	gormanii	micdadei	1	2
February 16, 1982												
Pipeline effluent	+	–	–	+	NA	NA	–	–	–	NA	NA	NA
March 22–23, 1982												
Trickling filter	+	–	–	+	NA	NA	–	+	+	NA	NA	NA
Pipeline effluent	+	–	–	–	NA	NA	–	+	+	NA	NA	NA
June 29–30, 1982												
Pipeline effluent	+	–	–	+	NA	NA	+	+	–	NA	NA	NA
Reservoir	+	–	–	+	NA	NA	–	+	–	NA	NA	NA
July 26–27, 1982												
Pipeline effluent[b]	–	–	+	+	+	+	+	+	+	+	+	+
Reservoir[b]	–	–	+	+	–	–	–	+	+	–	+	–

NA – conjugates not available.

[a] All species detected for samples collected on February 16, March 22–23, and June 29–30 came from guinea pig tissue

[b] Examination by direct fluorescent antibody staining of wastewater sample only.

The inability to consistently recover L. pneumophila from guinea pigs inoculated with up to 10^5 cfu of yolk-sac passed stock cultures suggests that there may be differences in susceptibility or that extremely high doses of Legionella are needed to infect some animals. The difference in lethal doses of Legionella pneumophila in egg-passed and agar-passed cultures, reported by McDade and Shepard (1979), suggests facultative differences in virulence factors and it is possible that the Legionella observed in Lubbock wastewater samples were relatively avirulent. It is also possible that these agents were nonviable, since isolates were not recovered from samples inoculated onto artificial media. The low levels of Legionella and high levels of non-Legionella present in the samples undoubtedly influenced the results. Isolation work using guinea pigs involves a trade-off between concentrating samples sufficiently to obtain infectious doses of Legionella consistently and diluting samples to nonlethal levels of other agents.

24-Hour Composite Samples--Human Enteric Viruses

The basic assay used for the quantitation of human viruses beginning in April 1981 allowed for the estimation of poliovirus levels in any sample taken as the difference between unaltered and poliovirus neutralized values enumerated on HeLa cell monolayers (see Tables P-1, P-2 and P-3 in Appendix P). In addition, extensive efforts were directed toward the identification of enteric viruses in selected wastewater samples. In some instances the presence of given viruses in sprayed wastewater was used in the selection of viral reagents for serological testing, especially if the study population showed a low level of immunity to the specific virus.

Specific viral identifications of environmental isolates are provided in Tables P-5 (in Appendix P), 25 and 26 (Lubbock wastewater), 27 (Hancock reservoir), and Tables P-6, P-7 and P-8 in Appendix P (Wilson wastewater). It should be noted that during the later portion of this study problems were encountered in the use of the Lim Benyesh-Melnick enterovirus typing pools in the RD cell line. Hence, isolates recovered as plaques on RD cell monolayers were not identified.

In addition to the expected recovery of all three polioviruses, selected coxsackie A and the first five coxsackie B viral serotypes were recovered during this study. Twenty recognized serotypes of echoviruses were also identified in wastewater samples. Not unexpectedly, seasonal occurrences of various human viruses were observed. This phenomenon was more pronounced in Lubbock wastewater, most likely due to the larger contributing population. The larger wastewater system also resulted in a greater diversity of viral types being recovered from Lubbock samples.

In general, poliovirus serotypes predominated during spring sampling, while coxsackie B viruses were more prevalent in the summer and fall. Polioviruses also reappeared in selected August-September samples, presumably reflecting preschool immunizations. Although echoviruses were found year round, most isolates were recovered during the summer months.

TABLE 25. VIRUSES ISOLATED FROM LUBBOCK PIPELINE EFFLUENT DURING 1982

Assay	Sampling Date Mar 8-9[a]	 Mar 22-23	 Apr 5-6	 Apr 19-20	 Jun 29-30	 Jul 26-27	 Sep 13-14
HeLa (unaltered concentrate)							
Concentration (pfu/L)	110	63	17	42	490	60	22
Virus type							
Polio 1	3	1		3		1	1
Polio 2	6	4	8	6			2
Polio 3	2	3	1	2		2	
Coxsackie B2							2
Coxsackie B4							1
Coxsackie B5				7	23	5	3
Echo 11	1	1					
Unidentified	6	1		2		1	2
TOTAL SAMPLED	18	10	9	20	23	9	11
HeLa (polio-neutralized)							
Concentration (pfu/L)	22	4.0	3.9	16	390	30	8.0
Virus Type							
Polio 3	1						
Coxsackie B5	1			5	11	6	4
Echo 1			1				
Echo 31	1						
Unidentified	6		1	1			
TOTAL SAMPLED	9		2	6	11	6	4
RD (polio-neutralized)							
Concentration (pfu/L)	<2	10	44	10	56	6.6	840
Virus type							
Coxsackie A16					1		
Coxsackie A19			2				
Coxsackie B5					1		
Echo 12			1		4		
Echo 15			1				
Unidentified			10		7	3	
TOTAL SAMPLED			14		13	3	

a Chlorination of wastewater effluent at treatment plant.

TABLE 26. VIRUSES ISOLATED FROM LUBBOCK PIPELINE EFFLUENT DURING 1983

Assay	Sampling Date Feb 16-17	Mar 21-22	Apr 18-19	Jul 11-12	Aug 8-9	Sep 12-13
HeLa (unaltered concentrate)						
Concentration (pfu/L)	44	31	100	280	120	56
Virus type						
Polio 1	3	1				
Polio 2	3	2			1	2
Polio 3	4		11			1
Coxsackie A13			1	1		
Coxsackie B2		1		2		
Coxsackie B3				4	10	11
Coxsackie B5		1		15	12	
Echo 25		1				
Unidentified	1	1	1			
TOTAL SAMPLED	11	7	13	22	23	14
HeLa (polio-neutralized)						
Concentration (pfu/L)	20	16	<4	300	130	180
Virus Type						
Coxsackie B2		2			1	
Coxsackie B3					10	13
Coxsackie B4					1	
Coxsackie B5		1			3	4
Unidentified	5	1			1	
TOTAL SAMPLED	5	4			16	17
RD (polio-neutralized)						
Concentration (pfu/L)				680		
Virus type						
Echo 19				7		
Unidentified	6	6		13	12	15
TOTAL SAMPLED	6	6		20	12	15

TABLE 27. VIRUSES ISOLATED FROM HANCOCK FARM RESERVOIRS DURING 1983

Assay	Sampling Date Feb 16-17	Mar 21-22	Apr 18-19	Jul 11-12	Aug 8-9	Sep 12-13
HeLa (unaltered concentrate)						
Concentration (pfu/L)	2		4			
Virus type						
Polio 2	1					
Coxsackie B5			1			
TOTAL SAMPLED	1	*	1	*	*	*

* No Isolates.

During the summer and fall of 1980 and 1983, coxsackie B3 and B5 viruses were present at high levels in Lubbock wastewater, while only coxsackie B5 predominated during the same period of 1981 and 1982. In Wilson sewage coxsackie B3 appeared at substantial levels only during 1980. Coxsackie B5 was prevalent in Wilson during the summer and fall of 1982 and 1983. The only elevated levels of coxsackie B2 observed during the course of environmental monitoring occurred in Wilson wastewater during the fall 1983.

Only two of the Hancock reservoir samples designated for viral identification analysis yielded viruses (Table 27). Poliovirus 2 and coxsackie B5 were recovered from this source during 1983.

24-Hour Composite Samples--Geometric Mean Data

To provide a basis of comparison between various irrigation seasons and to describe the potential microbial aerosol exposure during any given irrigation period, geometric means were computed for indicator organisms, viruses and physical-chemical analyses. Calculated values for Lubbock wastewater and Hancock reservoir water and Wilson wastewater are presented in Tables 28, 29 and P-9 (in Appendix P), respectively.

Comparing mean organism levels in the spring and summer of 1982 and 1983, one can see a substantially higher viral load in pipeline effluent during the second year of irrigation (see Table 28). Conversely, geometric mean data for Hancock reservoir samples collected during the summers of 1982 and 1983 suggest that once established the holding ponds produced effluent containing lower levels of fecal coliform and enteric viruses (see Table 29). Therefore, although the levels of microorganisms found in pipeline effluent increased during 1983, as shown in Figure 5, the actual aerosol load was reduced during the second year of irrigation since virtually all irrigated wastewater was drawn from the Hancock reservoirs.

30-Minute Composite Samples

Composite wastewater samples generally of 30 minutes duration were collected in 1982 during each microorganism, virus and quality assurance aerosol run and assayed for the microorganisms monitored in the aerosol, for enteroviruses, and for selected physical-chemical parameters. Results of these analyses are presented for pipeline wastewater during the irrigation in spring 1982 (Table P-10 in Appendix P) and summer 1982 (Table P-11 in Appendix P) and for reservoir wastewater during the summer 1982 irrigation (Table P-12 in Appendix P).

The 30-minute composite wastewater samples had similar values for all monitored parameters to those observed in the 24-hour composite samples for the same wastewater source. Thus, the aerosol sampling data should be representative of the microorganism levels in air generated by the irrigation system in 1982. Because aerosol sampling was conducted daily during some weeks, the 30-minute composite samples provide an indication of daily variability. The enterovirus level (5-day assay on HeLa cells) in the pipeline water was markedly elevated during the 2-day period when virus run V3 was

TABLE 28. GEOMETRIC MEAN OF MICROORGANISM CONCENTRATIONS IN LUBBOCK WASTEWATER

	Lubbock STP effluent						
	May/Jun 80	Summer 80	Fall-Win 80	Spring 81	May/Jun 81	Summer 81	Fall-Win 81
Sampling period	6-3	7-28	11-3/1-19	2-16/4-20	5-4/6-15	6-28/8-17	11-17/2-15
Number of samples	1	1	2	4	2	3	2
Bacteria (cfu/mL)							
Standard plate count	3,600,000	5,700,000	3,400,000	9,600,000		3,000,000	
Total coliforms	350,000	380,000	92,000	180,000	380,000	210,000	
Fecal coliforms	87,000	72,000	36,000	40,000	87,000	77,000	26,000
Fecal streptococci	4,700	2,000	5,100	6,900	1,100	4,600	11,000
Viruses (pfu/mL)							
Bacteriophage	1,400	3,200	1,500	1,600[a]		2,100[a]	900[a]
Enteroviruses							
HeLa, 5-day (uncorrected)	0.78	1.2	0.26	0.054	0.11	0.063	0.045
HeLa, polio-neutralized				0.018	0.020	0.018	0.001
RD, polio-neutralized				0.008	0.070	0.16	0.065
Physical Analyses (mg/L)							
Total organic carbon	83	40	115	142	70	92	117
Total suspended solids	96	78	199	158	74	53	114
Total volatile suspended solids	65	52	132	123	64	39	91
pH	6.5	6.6	7.1	7.2	7.0	7.0	7.2

continued...

TABLE 28. (CONT'D)

	Pipeline effluent						
Sampling period	Spring 82 3-1/4-26	May/Jun 82 6-14/6-29	Summer 82 7-26/9-13	Fall-Win 82 11-1/12-13	Spring 83 2-16/4-18	May/Jun 83 6-27	Summer 83 7-11/8-12
Number of samples	8	2	4	2	5	1	5
Bacteria (cfu/mL)							
Standard plate count			1,300,000				
Total coliforms	57,000		120,000	190,000	190,000		
Fecal coliforms	43,000	67,000	13,000	39,000	20,000	59,000	90,000
Fecal streptococci	3,400	2,000	880	1,400	4,100	1,200	160
Viruses (pfu/mL)							
Bacteriophage	560	840	180[b]		4,700[b]		
Enteroviruses							
HeLa, 5-day (uncorrected)	0.043	0.11	0.049	0.10	0.071	0.27	0.17
HeLa, polio-neutralized	0.009	0.10	0.026	0.041	0.018	0.14	0.20
RD, polio-neutralized	0.014	0.12	0.011	0.22	0.037	0.34	0.22
Physical Analyses (mg/L)							
Total organic carbon	105	65	61	54	67	42	30
Total suspended solids	149	92	78	91	72	35	26
Total volatile suspended solids	117	74	80	69	57	25	20
pH	7.3	7.2	7.5	7.5	7.6	7.6	7.5

a Based on a single sample.

b Based on two samples.

TABLE 29. GEOMETRIC MEAN OF MICROORGANISM CONCENTRATIONS IN HANCOCK RESERVOIR WASTEWATER

Sampling period Number of samples	May/Jun 82 6-14/6-29 2	Summer 82 7-26/9-13 4	Fall-Win 82 11-1/12-13 2	Spring 83 2-16/4-18 5	May/Jun 83 6-27 1	Summer 83 7-11/9-12 5
Bacteria (cfu/mL)						
Standard plate count		36,000				
Total coliforms		500	3,200	1,300		
Fecal coliforms	180	130	50	52	300	29
Fecal streptococci	8	3	2	54	10	1.9
Viruses (pfu/mL)						
Bacteriophage	16	0.85[a]		29[a]		
Enteroviruses						
HeLa, 5-day (uncorrected)	0.005	<0.002	0.010	<0.004	0.004	<0.004
HeLa, polio-neutralized	0.017	0.002	0.004	<0.004	<0.004	
RD, polio-neutralized	<0.009	0.004	0.006	<0.004	<0.004	<0.004
Physical Analyses (mg/L)						
Total organic carbon	26	22	28	26	17	25
Total suspended solids	121	27	50	29	11	27
Total volatile suspended solids	37	23	42	20	6	20
pH	7.7	8.1	8.4	8.5	8.2	8.9

[a] Based on two samples.

conducted (August 3-4, 1982). The polio-neutralized enterovirus assays indicate that over 90% of the enteroviruses in the pipeline wastewater on these days were polioviruses. Differential assays also indicate that the predominant enteroviruses in the sprayed pipeline wastewater were polioviruses during the spring 1982 irrigation and nonpolioviruses during the summer 1982 irrigation (except August 3-5), consistent with the 24-hour composite results. As expected, sporadic chlorination at the Lubbock treatment plant reduced bacterial indicator levels in the sprayed pipeline wastewater but had no apparent effect on enteric virus levels.

B. MICROORGANISM LEVELS IN AIR

Aerosol sampling data from the dye, particle size, background, microorganism, and virus runs follow. The sampling dates and meteorological conditions (Tables A-12 to A-16 in Appendix A), the sampler layouts (Figures 9 to 12), and the specific sampling conditions (Tables A-5 to A-10 in Appendix A) were previously presented for each of these runs. Data from the quality assurance runs were presented in Tables A-27 to A-29. A summary of sampled microorganism levels in air and inferences regarding downwind transport of aerosolized microorganisms are presented. Estimated distributions of AEI and other exposure measures are also presented.

Aerosolization Efficiency

One characteristic of a wastewater spray irrigation system that has a direct effect on exposure to aerosolized microorganisms is the aerosolization efficiency of the system. Aerosolization efficiency is defined as the proportion of the sprayed wastewater that forms droplets small enough to be carried downwind.

The aerosolization efficiency can be estimated through the use of a tracer dye. A measured amount of dye is injected into the wastewater of an operating spray rig. The concentration of dye in the air is measured at several points downwind of the rig. An atmospheric dispersion model is then used to estimate the dye concentration at each sampler location assuming complete aerosolization. The ratio of the measured concentrations to the calculated concentrations gives an estimate of the aerosolization efficiency.

Four dye runs were conducted to provide estimates of the aerosolization efficiency of the center pivot sprinkler system at the Hancock farm as operated during 1982 and 1983 (i.e., prior to the installation of spray nozzles which reduced aerosol production and drift). During injection of the Rhodamine dye, wastewater grab samples were collected at 1-minute intervals and assayed to determine the source strength of the dye. The dye concentrations in wastewater are presented in Table P-13 (Appendix P). Sampling was conducted only for minutes when dye was visible in the sprayed wastewater. The dye concentrations sampled in air are presented in Table P-14 (Appendix P). The lowest dye concentrations measured in air exceeded the method detection limit of 0.2×10^{-6} $\mu g/m^3$ by a factor of 2.

The dispersion model utilized was the Volume Source Diffusion Models Program (Cramer et al., 1972) that had been used to calculate the aerosolization efficiency of the Pleasanton, California, wastewater irrigation system (Anderson, 1977). Each spray nozzle was considered to be a separate volume source. The volume source parameters assigned were based on photographs of the operating spray rigs and on rig design data. The vertical dimension was estimated to be the nozzle height variation (0.6 m) plus the initial depth of the spray pattern (0.3 m). It was assumed that the spray rigs were designed with an overlap of approximately 100%. Therefore, the horizontal dimension of each volume source was set equal to the distance between the two nozzles immediately adjacent to the particular nozzle. These dimensions were divided by 4.3 to get the initial values of the standard deviations of the crosswind and vertical concentrations for each source. All sources were assumed to be at 1.8 m above the ground. Meteorological input parameters such as mean wind speed were obtained from field measurements at the run location and at the electronic weather station (Table A-15 in Appendix A). The standard deviation of the wind azimuth angle was set equal to the wind direction range divided by six. The standard deviation of the wind elevation angle was determined using the solar angle, the cloud cover and the wind speed. The effect of reflection from the top of the surface mixing layer was considered to be insignificant and was not included in the calculations.

An estimate of the concentration of dye that would have been measured at each receptor had all of the wastewater been aerosolized is presented in Table 30. The corresponding aerosolization efficiencies calculated for each of the samplers for each of the dye runs are also given in Table 30.

The aerosolization efficiency data are summarized in Table 31. The calculated aerosolization efficiency decreased with distance on each run, as expected, since some of the larger aerosols present at the nearer sampling distance should have settled out by the farther sampling distance. The median aerosolization efficiency over the four dye runs was 0.75% for the nearer samplers (25-40 m), 0.40% for the farther samplers (75-80 m), and 0.56% overall. This analysis indicates that about 0.40% of the nonvolatile materials in the wastewater escaped the Hancock farm spray zone as an aerosol during 1982 and 1983.

As Table 31 shows, the aerosolization efficiency values for the Hancock farm system in 1982 are about 50% to 100% larger than the corresponding median aerosolization efficiency values obtained for the Pleasanton, California irrigation system in 1977. This finding agrees with the visual impression that Hancock farm rigs appeared to be producing more aerosol. The median aerosolization efficiency was also higher for the Hancock farm system (0.56%) than for two other wastewater spray irrigation system which have been similarly evaluated (Camann, 1980): Fort Huachuca, Arizona (0.29%) and Deer Creek Lake State Park, Ohio (0.47%). Given the manner in which the Hancock farm spray nozzles deflected the wastewater upwards, it is not surprising to find a higher aerosolization efficiency for the Hancock spray system during the LISS, compared to other spray irrigation sites.

TABLE 30. CALCULATED CONCENTRATIONS AND CORRESPONDING AEROSOLIZATION EFFICIENCY POINT ESTIMATES FOR EACH SAMPLER DURING EACH DYE RUN

Run no.	Sampler position	Concentration ($\mu g/m^3$)		Aerosolization efficiency, %
		Calculated	Measured	
D1	3 Near	244	22	9.0
			4.5	1.8
	3 Far	141	0.38	0.27
			1.5	1.1
	5 Near	375	1.1	0.29
			0.89	0.24
	5 Far	191	1.1	0.58
			0.96	0.50
D2	4 Near	414	1.9	0.46
			7.5	1.8
	4 Far	235	2.3	0.98
			1.3	0.55
	6 Near	630	80	12.7
			0.46	0.07
	6 Far	361	0.67	0.19
			0.87	0.24
D3	4 Near	571	3.7	0.65
			0.47	0.08
	4 Far	326	1.9	0.58
			0.79	0.24
	6 Near	826	2.3	0.28
			9.7	1.2
	6 Far	495	0.71	0.14
			0.50	0.10
D4	3 Near	471	2.5	0.53
			2.4	0.51
	3 Far	261	1.0	0.38
			1.8	0.69
	5 Near	794	3.7	0.47
			6.3	0.79
	5 Far	436	1.3	0.30
			2.4	0.55

TABLE 31. SUMMARY OF AEROSOLIZATION EFFICIENCY OF THE HANCOCK FARM IRRIGATION SYSTEM IN 1982

	Geometric mean aerosolization efficiency, %		
Dye run number	Near pairs (25-40 m)	Far pairs (75-80 m)	Total
D1	1.04	0.54	0.75
D2	0.94	0.40	0.61
D3	0.36	0.21	0.28
D4	0.56	0.40	0.51
Hancock farm median (4 runs, 1982)	0.75	0.40	0.56
Pleasanton, CA[a] median (17 runs, 1976-77)	0.37	0.26	0.33

[a] See Camann, 1980.

Size of Viable Particles in the Wastewater Aerosol

The distribution of sizes of all the viable particles able to reproduce on standard plate count agar was determined upwind and at three downwind distances from the line of spray nozzles during irrigation with pipeline water using six-stage Andersen samplers. From these data, an estimate was made of the percentage of viable particles smaller than 5 µm, as this had been shown to be the range of efficient deposition in the human pulmonary system (Williamson, 1973). Larger particles (5-7 µm) can also be a factor, since they can enter the mouth and upper respiratory trace.

The data from the five particle size runs are presented in Table P-15 (Appendix P) and are summarized in Table 32. Fungal spores and aggregate organisms frequently yielded plates which could not be counted and were reported as TNTC (too numerous to count). In summarizing the sampling data, the reported TNTC values in Table P-15 (Appendix P) were inferred to have been large densities when the corresponding stage from the paired sampler and of adjoining stages were large, or as probable fungal contamination when these values were small.

The upwind viable particles had a relatively uniform distribution of particle diameters, with 52% below 4.7 µm. Spray irrigation of pipeline wastewater introduced a great number of large viable particles into the air, but few small viable particles. The density of all viable particles larger than 2 µm declined rapidly with increasing downwind distance. The density of smaller viable particles was largely unchanged with downwind distance. These patterns are consistent with gravitational settling of heavy low-energy particles and size reduction through drying or desiccation in the sprinkler aerosol. With these off-setting factors, a relatively constant percentage (38%-44%) of viable particles were smaller than 4.7 µm over the limited range of downwind distances investigated. Because both gravitational settling and size reduction through desiccation continue to operate in an off-settling manner well beyond 75 m downwind of pipeline

TABLE 32. STANDARD PLATE COUNT DENSITY OF VIABLE PARTICLES IN AIR BY DISTANCE AND PARTICLE SIZE

Andersen sampler stage	Range of particle sizes, μm	Geometric mean[a] standard plate count density in air by sampler distance			
			Downwind		
		Upwind	20-36 m	45-61 m	70-85 m
1	≥7.0	200	1,120	690	350
2	4.7-7.0	56	850	390	240
3	3.3-4.7	66	760	360	210
4	2.1-3.3	116	280	188	116
5	1.1-2.1	70	122	83	96
6	0.65-1.1	35	22	39	44
All	All	550	3,160	1,740	1,050
Percentage					
3-5	1.1-4.7	46%	37%	36%	40%
3-6	0.65-4.7	52%	38%	39%	44%

[a] Geometric mean over five particle size runs of the stage arithmetic means for the paired samplers.

irrigation, it is not possible to estimate the percentage of viable particles smaller than 5 μm in the downwind air at the much greater distances where most participants received their aerosol exposure.

The percentage of viable particles between 1.1 and 4.7 μm in the ambient upwind air at the Hancock farm between February and August 1982 (46%) was very similar to the 48-49% obtained by Bausum et al. (1983) at Deer Creek Lake State Park, Ohio, in July-August 1976 and the 42% reported by Bausum et al. (1982) at Fort Huachuca, Airzona, in October 1975. However, there was a marked difference among the three studies in the proportions of viable particles in this size range in the air downwind of the spray irrigation source. Bausum et al. consistently found that, compared to the upwind air, a much higher proportion (between 66% and 78%) of the viable particles were between 1 and 5 μm in the air from 21 m to 200 m downwind of the rectangular field source wastewater spray irrigation system at Deer Creek Lake. In marked contrast, they found that the proportion (43-50%) in this size range from 46 m to 152 m downwind of a single spray nozzle (a point source of wastewater aerosol) at Fort Huachuca was very similar to that in the upwind air. The LISS observed a slightly lower proportion (36% to 40%) in this size range downwind of the irrigation rig (a line source of pipeline wastewater aerosol) compared to the upwind air. The configuration of the wastewater aerosol source, the wastewater quality, the nozzle type, the operating conditions, and aerosol age may all be factors which affect the proportion of viable particles downwind of a spray irrigation aerosol source which are below 5 μm and can be efficiently deposited in the human pulmonary system.

Background Microorganism Densities in Ambient Air

The outdoor air near but in an upwind direction from the homes of eight participant households was monitored in summer before any irrigation commenced to measure ambient microorganism levels in the vicinity of homes. A ninth sampler was located downwind of the Wilson effluent pond to determine if it was a source of aerosolized microorganisms.

Four background air runs were conducted in nine locations in the study area before sunrise on the mornings of August 5 through August 8, 1980. A detailed description of the methodology, sampler locations and sampling conditions are contained in the Methods Section 4D. All runs were conducted at the same time of day (6:30-7:00 AM), same season, and with the same wind direction (from the south-southeast) to minimize sources of variability.

The sampled densities of the standard plate count, fecal coliforms, fecal streptococci, mycobacteria, and coliphage in the ambient air during the four background runs are presented in Table P-16 (Appendix P). The Wilson effluent pond does not appear to have been an appreciable source of aerosolized microorganisms. Geometric means calculated over the four runs are provided in Table 33 to estimate background microorganism levels in the ambient air just upwind of homes.

Fecal coliforms were only detected in 1 of the 30 air samples near homes (at location F). Assuming there was a constant background level near homes throughout the study area, this background level of fecal coliforms is estimated as 0.01 cfu/m^3. As anticipated, no coliphage were detected in the 30 air samples near homes, yielding a coliphage background level below 0.005 pfu/m^3. Mycobacteria were detected in 9 of the 30 air samples near homes for an estimated background level of 0.05 cfu/m^3. Standard plate count, monitored as a positive control, indicated that background bacterial concentrations in the air near homes was about 450 cfu/m^3.

Fecal streptococci were prevalent in these background air samples and were found in 27 of the 30 air samples near homes, at concentrations ranging from 0.1 cfu/m^3 to 11 cfu/m^3. Geometric mean air concentrations of fecal streptococci ranged from about 0.2 cfu/m^3 at locations D, E, G and H to 2 cfu/m^3 at location A. The Wilson sites (0.87 cfu/m^3 geometric mean) appear to have differed from the rural sites (0.32 cfu/m^3 geometric mean), with locations A, C and F having higher air levels of fecal streptococci than the other locations.

The sources of the aerosolized fecal streptococci and mycobacteria are unknown. It is possible that these organisms adhered to dust or particulates, since soil samples were found to contain fecal streptococci. The prevalence and wide distribution of fecal streptococci densities in air between about 0.1 cfu/m^3 and 1 cfu/m^3 suggests a normal background of this order of magnitude throughout the study area. Further, there is no known feed lot or similar operation south or southeast of the Wilson area which might produce the observed effect. High air levels of fecal streptococci were observed consistently at locations A and F and occasionally at C. Twelve of the fecal streptococci colonies from the first air sample at

TABLE 33. GEOMETRIC MEAN MICROORGANISM DENSITIES IN AMBIENT AIR SAMPLED ON BACKGROUND RUNS[a]

Sampler location[b] (near participant home)	Background microorganism concentration in air				
	Standard plate count (cfu/m^3)	Fecal coliforms (cfu/m^3)	Fecal streptococci (cfu/m^3)	Mycobacteria (cfu/m^3)	Coliphage (pfu/m^3)
Wilson-A	750	<0.03	2	<0.03	<0.04
Wilson-B	700	<0.04	0.3	0.04	<0.04
Wilson-C	430	<0.04	1.1	0.07	<0.04
Wilson effluent pond-D	390	<0.04	0.2	0.4	<0.04
Rural (Hancock)-E	500	<0.04	0.2	0.04	<0.04
Rural (NE)-F	510	0.09	1.5	<0.03	<0.04
Rural (SE)-G	150	<0.04	0.2	0.07	<0.04
Rural (SW)-H	510	<0.04	0.2	0.04	<0.04
Rural (NW)-I	390	<0.04	0.3	0.2	<0.04
Wilson (geometric) mean	610	<0.01	0.87	0.04	<0.012
Rural (geometric) mean	380	0.02	0.32	0.06	<0.008
Estimated area background (A-I, excluding D, geometric mean)	450	0.01	0.47	0.05	<0.005

NOTE: < indicates none detected in any samples at this location.

[a] Conducted in August 1980.
[b] Sampler locations shown in Figure 8.

location C (8 cfu/m^3) were characterized: four were classified as S. durans, which may be of human origin, and eight were categorized as S. bovis or S. equinus, which are more likely of animal than human origin. A plausible hypothesis is that the passage of air through Wilson elevates the levels of aerosolized fecal streptococci of both human and animal origin. The data at location F suggest there also are comparable isolated local sources in some rural areas.

A high level of mycobacteria (3.4 cfu/m^3) was observed on the fourth air sample taken downwind of the Wilson effluent pond (location D); cows were grazing approximately 300 to 500 m upwind during this sampling. Representative mycobacteria colonies from this sample were speciated. All isolates tested belonged to the ''M. avium complex,'' consisting of M. avium and M. intracellulare, of Runyon group III. Traditionally, these species are the major disease-associated strains of Runyon group III and hence are classified as pathogens.

The background densities of fecal coliforms and fecal streptococci in the ambient air were similar to those obtained by Jones and Cookson (1983) in a Washington, D.C. suburban area over a 24-month monitoring period. Whereas the LISS obtained ambient geometric mean fecal coliform densities of <0.01 cfu/m^3 for Wilson and 0.02 cfu/m^3 for the rural study area, Jones and Cookson did not detect fecal coliforms in their suburban study area in 225 m^3 of ambient air (<0.004 cfu/m^3). The LISS ambient geometric mean fecal streptococci densities were 0.87 cfu/m^3 for Wilson and 0.32 cfu/m^3 for the rural area. In the Washington, D.C. suburban area, the 95% confidence intervals for the mean fecal streptococci density were 0.20 to 0.43 cfu/m^3 in 1979 and 0.30 to 0.55 cfu/m^3 in 1980, including the winter samples in which no fecal streptococci were recovered. The Washington, D.C. suburb had significantly higher densities of airborne bacterial particles in summer and fall (especially September) than in the winter and spring months.

Microorganism Densities in Downwind Air from Microorganism Runs

The densities of microorganisms in the air upwind and at four distances downwind from the irrigation nozzle line were determined simultaneously in each of 20 microorganism runs. The wastewater density and the sampled densities in air of fecal coliforms, fecal streptococci, mycobacteria, Clostridium perfringens, and coliphage are presented, respectively, in Tables P-17 through P-21 of Appendix P. These data are summarized in Table 34 by microorganism group, source of wastewater and irrigation season. Some caution must be exercised in interpreting Table 34 since the estimated densities were based on widely varying numbers of air samples and since environmental conditions were not represented equivalently in the various distance categories. Nevertheless, Table 34 does provide a good overview of the extensive air sampling data.

Statistical tests were conducted comparing the downwind and upwind aerosol data to confirm that the Hancock farm irrigation system was a significant source of aerosolized microorganisms. The results shown in Table 35 indicate that irrigation with pipeline wastewater was a significant

TABLE 34. ESTIMATED DENSITIES SAMPLED ON MICROORGANISM AND VIRUS AEROSOL RUNS[a]

		Microorganism concentration, geometric mean[b]						
			Air[a] (no./m^3 air)					
				Downwind of irrigation nozzle line (m)				
Microorganism group	Source-season	Wastewater (no/mL)	Upwind	25-89	90-149	150-249	250-349	350-409
Fecal coliforms (cfu)								
	Pipeline-spring 1982	109,000	<0.01	180	6	3	4[c]	
	Pipeline-summer 1982	18,500	<0.01	200[d]	2	2	0.8	0.5
	Reservoir-summer 1982	320	<0.03	2	0.2	0.6	<0.2[c]	<0.2[c]
Fecal streptococci (cfu)								
	Pipeline-spring 1982	5,700	0.08	140	38	23	20[c]	
	Pipeline-summer 1982	1,310	0.2	200[d]	5	5	0.7	0.6
	Reservoir-summer 1982	11	0.04	0.04	0.2	0.2	0.3[c]	0.2[c]
Mycobacteria (cfu)								
	Pipeline-spring 1982	21,000	0.2	8	2.1	0.9	4[c]	
	Pipeline-summer 1982	24,000	0.07	0.4[d]	0.6	0.08	0.2	0.1
	Reservoir-summer 1982	100	<0.02	0.08[d]	0.1[d]	0.06[d]	<0.05[c]	<0.07[c]
Clostridium perfringens (cfu)								
-Vegetative	Pipeline-1982	270	0.09	9[c]	2[d]	2[d]	1[d]	0.9[d]
	Reservoir-summer 1982	3	<0.2[c]	<0.07	<0.2[d]			
-Sporulated	Pipeline-summer 1982	210	<0.04[d]		0.5[d]	1[d]	0.4[d]	0.3[d]
	Reservoir-summer 1982	<1	<0.2[c]	<0.07[d]	<0.2[c]			
Coliphage (cfu)								
	Pipeline-spring 1982	1,060	<0.01	11	4	2	0.9[c]	
	Pipeline-summer 1982	630	0.3	7[d]	1	0.7	0.1	0.07
	Reservoir-summer 1982	2.5	<0.01	0.03	0.06	0.07	0.06[c]	0.06[c]
Enteroviruses (pfu)								
-HeLa cells	Pipeline-1982	0.22		0.048[d]				
-RD cells	Pipeline-1982			0.050[d]				

<X - Detection limit of pooled samples when none detected in each sample.

a Excludes samples probably contaminated.
b In geometric mean calculation, >C ⟶ 2C and <C ⟶ C/2, where C is aerosol concentration.
c Based on one to two air samples.
d Based on three to six air samples.

TABLE 35. CONFIRMATION OF SPRAY IRRIGATION OF PIPELINE WASTEWATER AS A SIGNIFICANT SOURCE OF MICROORGANISMS IN DOWNWIND AIR: PAIRED DOWNWIND VS. UPWIND DENSITIES

	Significant increases in mean microorganism density in air at sampled downwind distance?[a] (p-value)[b]			
	90–149 m Downwind	150–249 m Downwind	250–349 m Downwind	350–409 m Downwind
Fecal coliforms[c]	Yes (0.002)	Yes (0.002)	Maybe[d] (0.06)	Insufficient data
Fecal streptococci	Yes (<0.0005)	Yes (<0.0005)	Yes (0.02)	Maybe[d,e] (0.06)
Mycobacteria	Yes (<0.0005)	Yes (0.05)	Maybe[d] (0.08)	No (>0.25)
Coliphage[c]	Yes (0.002)	Yes (0.01)	Maybe[d] (0.06)	Insufficient data

[a] Yes if $p<0.05$
Maybe if $0.05<p<0.10$
No if $p>0.10$

[b] One-sided t-test of difference in population means for paired (downwind-upwind) observations; ln (microorganism air density from average of sampler pair) transformation of each observation used to reduce variance inequality.

[c] Signed rank test employed for all distances because of highly skewed distribution of paired differences.

[d] Lack of significance may be result of insufficient paired observations.

[e] Significant increase using one-sided t-test of difference in two independent population means.

source of the monitored microorganisms to at least the following downwind distances:

Fecal coliforms	at least 200 m
Fecal streptococci	at least 300 m
Mycobacteria	at least 200 m
Coliphage	at least 200 m

Although insufficient data existed for statistical testing, pipeline irrigation also appeared to be a source of *Clostridium perfringens* to at least 200 m downwind (see Table P-20 of Appendix P).

These air data provide convincing evidence that spray irrigation of wastewater directly from the pipeline was a substantial source of each of the monitored microorganism groups under most conditions of actual operation of the irrigation system at the Hancock farm. The air densities within 100 m downwind of pipeline irrigation were markedly elevated above upwind levels, ranging from two orders of magnitude elevation for mycobacteria to four or more orders of magnitude elevation for fecal coliforms. Under some conditions of operation, particularly at night or at high wind speeds (>7 m/sec), sprinkler irrigation of pipeline wastewater appeared to elevate the ambient (upwind) density in air of fecal coliforms, fecal streptococci, *Clostridium perfringens*, and coliphage beyond 400 m downwind and of mycobacteria to about 300 m downwind.

Irrigation with wastewater which had been stored in a reservoir produced much lower microorganism levels in air than did irrigation with pipeline wastewater. Nevertheless, the air sampling data do demonstrate that irrigation with wastewater stored in Reservoir 1 also was a source of aerosolized fecal coliforms, fecal streptococci and coliphage. These organisms were frequently detected at 125 m downwind and may occasionally have been carried more than 200 m from rigs irrigating with reservoir wastewater.

The aerosolized fecal coliforms exhibited more rapid die-off than did the other monitored microorganism groups. The aerosol data are consistent with the hypothesis that a large proportion of the aerosolized colony forming units of each microorganism were vulnerable and were rapidly inactivated after aerosolization, while the remaining (hardy or protected) organisms survived without detectable die-off out to the farthest distances sampled.

Microorganism densities in air downwind of spray irrigation with pipeline and reservoir wastewater at the Hancock farm are contrasted in Table 36 with densities downwind from other wastewater aerosol sources (both spray irrigation sites and aeration basins of activated sludge sewage treatment plants). The geometric mean densities of fecal coliforms, fecal streptococci and coliphage downwind of Hancock farm irrigation with pipeline wastewater were at least one or two orders of magnitude higher than at the other sites. However, downwind mycobacteria densities were comparable or lower. Microorganism densities downwind of reservoir wastewater irrigation at the Hancock farm were comparable or lower than at the other sites.

TABLE 36. MICROORGANISM DENSITIES IN AIR AT HANCOCK FARM COMPARED TO OTHER WASTEWATER TREATMENT FACILITIES (USEPA, 1982)

	Geometric mean microorganisms/cubic meter[a]				
	Spray irrigation			Aeration basin	
	Hancock farm Wilson, TX		Pleasanton	Schaumburg	Tigard
Microorganism Distance downwind	Pipeline	Reservoir	CA	IL	OR
Fecal coliforms					
Upwind	<0.006[b]		0.04	0.2	ND[c]
10-30 m	ND	ND	2.1	0.7	ND
31-80 m	180	2	1.0	0.5	ND
81-200 m	3	0.4	0.5	0.3	ND
Fecal streptococci					
Upwind	0.07		0.5	<2	0.06
10-30 m	ND	ND	3.0	<2	5.0
31-80 m	150	0.4	1.3	15	2.7
81-200 m	20	0.3	0.9	<2	1.5
Mycobacteria					
Upwind	0.1		0.4	ND	<0.02
10-30 m	ND	ND	ND	ND	28
31-80 m	2.1	0.08	3.6	ND	15
81-200 m	0.8	0.10	1.6	ND	5
Coliphage					
Upwind	<0.003		0.02	0.02	<0.04
10-30 m	ND	ND	0.7	0.08	2.3
31-80 m	10	0.03	0.08	0.04	1.1
81-200 m	2	0.07	0.4	<0.04	0.06
Enteroviruses					
40-65 m	0.05	ND	0.006	<0.02	<0.002

a Colony forming units (cfu) per m^3 for bacteria; plaque forming units (pfu) per m^3 for viruses.

b < = None detected in any samples, yielding the stated cumulative detection limit.

c ND - no data available--sampling and analysis not performed for this microorganism or distance.

Enterovirus Densities in Downwind Air from Virus Runs

Four special virus runs were conducted to estimate enterovirus levels in the air downwind from irrigation nozzles spraying pipeline wastewater. The indigenous enterovirus levels ranged from 0.066 to 2.2 pfu/mL of sprayed wastewater during these four runs, conducted on March 16, 1982 (Table P-9) and August 2, 4 and 24, 1982 (Table P-10). As shown in Table 37, enteroviruses were recovered from the aerosol samples' concentrate on every virus run and at similar concentrations on the HeLa and RD cell lines.

TABLE 37. VIRUSES[a] RECOVERED FROM AEROSOL SAMPLES DURING VIRUS RUNS

	Virus runs							
	V1 (3-16-82)		V2 (8-2-82)		V3 (8-4-82)		V4 (8-24-82)	
Cell line	pfu/mL	Total expected pfu[b]	pfu/mL	Total expected pfu[b]	pfu/mL	Total expected pfu[b]	pfu/mL	Total expected pfu[b]
HeLa	0.057 (2 pfu)	4	0.20 (3 pfu)	14	310	22,000	0.38 (5 pfu)	16
RD	0.029 (1 pfu)	2	0.32 (9 pfu)	22	350	25,000	0.31 (9 pfu)	22

[a] Based on confirmed isolates.

[b] 70 mL of concentrate from each aerosol run (V1: 3416 mL concentrated; V2: 2380 mL; V3: 2690 mL; V4: 2790 mL). Total number of plaques expected if all 70 mL of concentrate were plated on a single cell line.

The sampled enterovirus densities in wastewater and air are presented in Table 38 and compared to those obtained in 1977 in the two virus runs at the Pleasanton, California, wastewater irrigation system. The range of enterovirus densities in air observed on three of the LISS virus runs (0.002 to 0.015 pfu/m^3) at 46 to 60 m downwind are comparable to those observed at 63 m downwind of the Pleasanton sprinkler line.

During Virus Run V3 conducted on August 4, the enterovirus density was elevated in the wastewater sample to 2.2 pfu/mL. However, the enterovirus density in air at 44 m downwind was exceptionally elevated in the aerosol sample to a level (17 pfu/L) only one order of magnitude below those generally observed for the indicator bacteria (see Table 34). The degree of anomaly is indicated in Table 38 by the ratio of aerosol to wastewater density of 7.4 for Run V3, compared to ratios ranging from 0.02 to 0.15 for the other five virus runs. The majority of the aerosolized enteroviruses sampled on Run V3 appear to have been poliovirus 1, based on neutralization with monovalent antiserum. Since poliovirus 1 was used in the laboratory to determine concentration efficiency, a thorough evaluation of laboratory procedures was conducted. The evaluation indicated that laboratory handling of aerosol-related samples had not compromised their integrity. Field contamination of the Run V3 aerosol sample is not a plausible hypothesis because the aerosol sample contained more plaque forming units than 10 liters of the wastewater and because there was no indication of any irregularity

TABLE 38. SAMPLED ENTEROVIRUS DENSITIES ON VIRUS RUNS

Virus run Date	Distance from spray line (m)	Cell line	Enterovirus density in wastewater pfu/mL	Enterovirus density in air pfu/m^3	Ratio of aerosol density to wastewater density
Lubbock Infection Surveillance Study					
V1 3-16-82	60	HeLa RD	0.16	0.0029 0.0015	0.018
V2 8-2-82	46	HeLa RD	0.10	0.011 0.018	0.11
V3 8-4-82	44	HeLa RD	2.2	16.2 18.3	7.4
V4[a] 8-24-82	49	HeLa RD	0.066	0.010 0.013	0.15
Pleasanton Aerosol Monitoring Study[b]					
V2-I 2-26-77	63	HeLa (5d)	0.036	0.0047	0.13
V2-II 4-9-77	63	HeLa (5d)	0.18[c]	0.0070	0.039

a Pipeline wastewater chlorinated at Lubbock SeWRP at rate of 500 lb/day.
b From Johnson et al., 1980.
c Geometric mean of UTA and UTSA values.

in the field sampling. Hence, there is no laboratory or field evidence of contamination to cast doubt on the validity of the anomalously high enterovirus density in air obtained on Run V3.

The identification of viral isolates recovered from the wastewater and from the aerosol during the virus runs are presented in Table 39. The specific viruses found in the aerosol sample were nearly always also recovered from the wastewater being sprayed at the time, despite differences in procedures used on the wastewater and aerosol samples. Quantitative interpretation of Table 39 is difficult, because the stability of various enteroviruses in the aerosol may differ.

The virus runs clearly established that spray irrigation with pipeline wastewater at the Hancock farm was a substantial source of aerosolized enteroviruses in both the spring 1982 and summer 1982 irrigation periods. The geometric mean enterovirus density in air was 0.05 pfu/m^3, although a much higher density (17 pfu/m^3) was sampled on one run in August 1982. It can be inferred from their relative enterovirus concentrations in the wastewater (see Table 21) that irrigation with reservoir wastewater produced a much lower enterovirus density in the air downwind of the irrigation rig than did the sampled irrigation with pipeline wastewater.

TABLE 39. IDENTIFICATION OF VIRAL ISOLATES RECOVERED DURING VIRUS RUNS

Source of isolates	Virus run V1		Virus run V2		Virus run V3[a]		Virus run V4	
	Virus	No. of isolates	Virus	No. of isolates	Virus	No. of isolates	Virus	No. of isolates
Aerosol	Polio 2	2	Polio 2	1	Polio 1	1[a]	Polio 1	8
	Polio 3	1	Cox B5	1	Polio 2	18	Polio 2	2
	TOTAL	3	Unidentified	10	Polio 3	22	Echo 13	1
			TOTAL	12	Cox B5	1	Unidentified	3
					Echo 11	1	TOTAL	14
					Unidentified	11		
					TOTAL	54		
Wastewater	Polio 2	4	Polio 3	1	Polio 1	1[a]	Polio 1	3
	Polio 3	4	Cox A16	1	Polio 2	3	Polio 2	3
	Cox A9	3	Cox B5	24	Polio 3	2	Cox B2	1
	Echo 5	1	Echo 11	1	Cox B5	30	Cox B5	18
	Echo 11	1	Echo 12	1	Echo 11	1	Echo 16	1
	Echo 13	1	Unidentified	4	Echo 12	1	Echo 24	1
	Echo 17	1	TOTAL	32	Echo 24	1	Echo 25	1
	Echo 19	2			Echo 25	1	Echo 33	1
	Echo 20	1			Unidentified	15	Unidentified	7
	Echo 21	2			TOTAL	55	TOTAL	36
	Echo 25	2						
	Echo 27	1						
	Unidentified	8						
	TOTAL	31						

[a] The majority of the aerosol plaques (94%) were polio 1 based on neutralization with monovalent antiserum. Only plaques picked from polio 1 neutralized aliquots were selected for identification using enterovirus pools.

As Table 36 illustrates, the enterovirus density in air downwind of irrigation with pipeline wastewater at the Hancock farm was an order of magnitude higher than at the Pleasanton, California, spray irrigation site. It was also much greater than downwind of the aeration basins at monitored sewage treatment plants.

Microorganism Exposure Via the Wastewater Aerosol

The increased exposure to aerosolized microorganisms which LISS participants experienced while within 400 m downwind of a Hancock farm irrigation rig can be inferred from the air sampling data. In Table 40, the microorganism levels in air downwind of an irrigation rig utilizing wastewater from the pipeline or a reservoir are contrasted with the densities of these same microorganism groups in the ambient outdoor air in fields and just upwind of participants' homes. Aerosol densities downwind of the irrigation nozzle line were determined for both pipeline and reservoir sources of wastewater from the 20 microorganism runs and four virus runs.

Ambient background densities of the monitored microorganisms in the air just upwind of eight participant homes were determined in the four background runs at dawn in early August 1980 prior to irrigation or construction activities. Ambient background densities in the fields were estimated from the upwind samplers from 18 of the 20 microorganism runs in 1982 in which there was no operating irrigation rig or nearby human activity upwind of the upwind samplers. Ambient background levels of the bacterial indicators, especially fecal streptococci, were higher near homes than in the fields. Mycobacteria and vegetative Clostridium perfringens were also present in the ambient air, both with an average level in the fields of about 0.1 cfu/m^3. As expected, coliphage was not found in the ambient air near homes or in fields.

The microorganism densities in air downwind of irrigation with pipeline wastewater were from two to at least four orders of magnitude higher than in the ambient background air outside of participants' homes. Statistical tests established (see Table 41) that the downwind levels were significantly higher than the background levels in ambient air outside the homes of participants: fecal coliform levels to beyond 400 m downwind, mycobacteria and coliphage levels to at least 300 m downwind, and fecal streptococci levels to at least 200 m downwind.

The more highly exposed LISS participants received substantial doses of microorganisms from the wastewater aerosol during four major periods of wastewater irrigation at the Hancock farm. All of the irrigation wastewater was obtained via pipeline directly from the Lubbock SeWRP in the spring 1982 irrigation period, since operation of the reservoirs had not been approved at that time. Pipeline wastewater comprised 64%, 0% and 1%, respectively, of the total applied by spray irrigation in the summer 1982, spring 1983 and summer 1983 irrigation periods. Since microorganism densities were much higher in the wastewater from the pipeline than from the reservoirs, the exposure which most of the study population received to most microorganisms via the wastewater aerosol was greater in 1982 than in 1983.

TABLE 40. ESTIMATED MICROORGANISM DENSITIES IN AIR DOWNWIND OF IRRIGATION IN 1982 RELATIVE TO AMBIENT BACKGROUND LEVELS NEAR HOMES AND IN FIELDS

	Microorganism concentration in air[a] (no./m^3)				
	Ambient background		Downwind of irrigation line[b]		
Microorganism group/ Wastewater source	Homes[c]	Fields[d]	20-89 m	90-249 m	250-409 m
Fecal coliforms (cfu)	0.01	<0.006			
Pipeline			180	3	0.8
Reservoir			2	0.4	<0.08
Fecal streptococci (cfu)	0.5	0.07			
Pipeline			150	20	1
Reservoir			0.4	0.3	~0.3
Mycobacteria (cfu)	0.05	0.1			
Pipeline			2.1	0.8	0.3
Reservoir			0.08	0.10	<0.03
Clostridium perfringens (cfu)					
- Vegetative		0.08			
Pipeline			~9	2	1
Reservoir			<0.07	<0.2	
- Sporulated		<0.03			
Pipeline				0.8	0.3
Reservoir			<0.07	<0.2	
Coliphage (cfu)	<0.005	<0.003			
Pipeline			10	2	0.13
Reservoir			0.03	0.07	~0.06
Enteroviruses[e] (pfu)					
Pipeline			0.05		

a Geometric mean from aerosol sampling.

b From 20 microorganism runs.

c From background runs.

d From upwind samplers for 18 microorganism runs with no upwind rig in operation and no nearby human activity.

e From four virus runs.

TABLE 41. SIGNIFICANT ELEVATION OF MICROORGANISM DENSITY IN AIR DOWNWIND OF SPRAY IRRIGATION WITH PIPELINE WASTEWATER RELATIVE TO AMBIENT BACKGROUND OUTSIDE PARTICIPANT HOMES

	Significant increases in mean microorganism density in air downwind vs. mean background run level at homes?[a] (p-value)[b]			
	90-149 m Downwind	150-249 m Downwind	250-349 m Downwind	350-409 m Downwind
Fecal coliforms	Yes (<0.0005)	Yes (<0.0005)	Yes (<0.0005)	Yes (<0.0005)
Fecal streptococci	Yes (<0.0005)	Yes (<0.0005)	No (0.11)	No (>0.25)
Mycobacteria	Yes (<0.0005)	Yes (0.001)	Yes (<0.0005)	Maybe[c] (0.07)
Coliphage	Yes (<0.0005)	Yes (<0.0005)	Yes (0.04)	No (0.25)

[a] Yes if $p<0.05$
Maybe if $0.05<p<0.10$
No if $p>0.10$

[b] One-sided t-test of difference in means in two independent populations; ln (microorganism air density from average of sampler pair) transformation of each observation used to reduce variance inequality.

[c] Lack of significance may be result of insufficient observations at 350-409 m downwind.

The relative ranking of the four irrigation periods with regard to cumulative seasonal dose of microorganisms received by participants from the air can be inferred at a given distance from the Hancock farm from the sampling and wastewater application data. A relative aerosol exposure measure, RAEM, was constructed to provide the basis for ranking. RAEM is calculated for a given microorganism group, a given irrigation period, and a given downwind distance (d) by accumulating its component values for pipeline irrigation and reservoir irrigation, as

$$\text{RAEM} = \left[\left(\frac{A_{as}(d)}{W_{as}}\right) W_c \cdot V\right]_{\text{pipeline}} + \left[\left(\frac{A_{as}(d)}{W_{as}}\right) W_c \cdot V\right]_{\text{reservoir}}$$

where $A_{as}(d)$ - microorganism concentration in air at distance d on aerosol sampling (as) runs (from Table 34)
W_{as} - microorganism concentration in wastewater on aerosol sampling runs (from Table 34)
W_c - microorganism wastewater concentration in 24-hour composites (c) during the irrigation period (from Tables 28 and 29)
and V - average wastewater irrigation volume, cm (from Table 4)

The RAEM values for the monitored microorganism groups are presented in Table 42 by irrigation period and downwind distance. The RAEM values provide a ranking of the four irrigation periods regarding cumulative exposure via the wastewater aerosol to each monitored microorganism group at a constant downwind distance. Consider, for example, exposure at 150-249 m downwind, the farthest distance range at which air sampling was regularly conducted to determine microorganism densities in air. The irrigation periods in which the cumulative microorganism dose in air at 150-249 m downwind can be inferred from RAEM in Table 42 to have been largest and second largest were:

	Irrigation period by rank	
	1 Largest exposure	2 Second largest exposure
Fecal coliforms	Summer 1982	Spring 1982
Fecal streptococci	Spring 1982	Summer 1982
Enteroviruses (at 44-60 m)	Summer 1982	Summer 1983

It appears reasonable to extrapolate the relative seasonal exposure to microorganisms in the wastewater aerosol from the distances in Table 42 to the distance of the residences of the more highly exposed study population (approximately ≤600 m for AEI>5 and ≤800 m for AEI>3). For each of the microorganism groups with adequate aerosol and wastewater monitoring data, extrapolation from Table 42 indicates that summer 1982 was the irrigation period when most of the more highly exposed LISS participants received either their largest or their second largest cumulative dose of the microorganism group from the wastewater aerosol. In particular, the cumulative enterovirus dose received from the wastewater aerosol was probably at least an order of magnitude larger during summer 1982 than during any other irrigation period.

Estimates of Aerosol Exposure Index (AEI) and Other Participant Exposure Measures

Aerosol Exposure Index--

The aerosol exposure index (AEI) is a measure of the degree of a participant's cumulative exposure to microorganisms in the wastewater aerosol, relative to all other study participants, during a given irrigation period. The procedure for calculating an estimate of AEI for each participant in each irrigation period was provided in Section 4C.

The distribution of AEI values of all participants is presented in Table 43 for each of the four major irrigation periods. By design, the AEI percentile distribution is similar for each irrigation period. Thus, a participant's AEI value ranks his aerosol exposure relative to all other participants within that irrigation period. However, one cannot compare AEI values across irrigation periods because the number of pathogens emitted in aerosol form varied from one period to another (see Table 42). The relevant factors, including the volumes of wastewater applied from pipeline

TABLE 42. RELATIVE AEROSOL EXPOSURE MEASURE (RAEM) TO SPRAYED MICROORGANISMS BY IRRIGATION PERIOD AND DOWNWIND DISTANCE

		Relative aerosol exposure measure, RAEM[a]			
	Downwind distance, m	Spring 1982	Summer 1982	Spring[b] 1983	Summer[b] 1983
Fecal coliforms	25-89	410	970	4.8	200
	90-149	14	10	0.5	2.2
	150-249	7	11	1.4	2.7
	250-349	~9	~4	<0.5	~0.9
	350-409		~2.5	<0.5	~0.6
Fecal streptococci	25-89	490	930	30	5.9
	90-149	130	23	15	0.6
	150-249	80	23	15	0.6
	250-349	~70	3.5	~20	~0.8
	350-409		3.0	~15	~0.5
Mycobacteria	25-89	40	0.7		
	90-149	10	1.0		
	150-249	4	0.4		
	250-349	~20	~0.3		
	350-409		~0.2		
Coliphage	25-89	34	14	0.5	
	90-149	12	2.1	1.0	
	150-249	6.2	1.5	1.2	
	250-349	~3	0.3	~1.0	
	350-409		0.2	~1.0	
Enteroviruses	44-60	0.004	1.5	0.0004 or 0.006[c]	0.04 or 0.02[d]

[a] RAEM is based on microorganism concentrations in air $A_{as}(d)$ and wastewater W_{as} on aerosol sampling (as) runs, in 24-hour composites (c) during irrigation period (W_c), and average wastewater irrigation volume (V) sprayed:

$$\mathrm{RAEM} = \left[\left(\frac{A_{as}(d)}{W_{as}}\right) W_c \cdot V\right]_{\text{pipeline}} + \left[\left(\frac{A_{as}(d)}{W_{as}}\right) W_c \cdot V\right]_{\text{reservoir}}$$

[b] Based on A_{as} and W_{as} values from 1982 aerosol sampling runs for corresponding season.

[c] Values based on A_{as}/W_{as} ratios from spring 1982 and all virus air sampling runs, respectively.

[d] Values based on A_{as}/W_{as} ratios from summer 1982 and all virus air sampling runs, respectively.

TABLE 43. DISTRIBUTION OF PARTICIPANT AEROSOL EXPOSURE INDEX (AEI) BY IRRIGATION PERIOD

	Irrigation period						
	1 Spring 1982	2 Summer 1982	3 Spring 1983	4 Summer 1983	5 1982	6 1983	7 1982 1983
NO. (%) OF PARTICIPANTS	387	369	335	315	365	314	305
AEI Percentile Distribution							
Minimum	0	0	0	0	0	0	0
10 %ile	0.06	0.05	0.08	0.09	0.07	0.10	0.07
25 %ile	0.31	0.38	0.51	0.64	0.52	0.81	0.69
50 %ile	2.01	1.68	2.12	1.77	1.75	1.93	1.74
75 %ile	3.55	2.89	3.79	2.79	2.96	3.00	2.85
90 %ile	6.55	7.80	7.80	10.15	7.44	8.95	5.95
Maximum	82.26	149.22	82.84	151.17	139.29	120.53	138.67
By Exposure Groups							
#Low (AEI <3)	260(67)	287(78)	218(65)	248(79)	277(76)	234(75)	241(79)
#High (AEI≥3)	127(33)	82(22)	117(35)	67(21)	88(24)	80(25)	64(21)
By Exposure Levels							
#Low (AEI<1)	119(31)	124(34)	97(29)	98(31)	118(32)	91(29)	97(32)
#Intermed. (1-5)	222(57)	202(55)	193(58)	175(56)	203(56)	180(57)	172(56)
#High (AEI>5)	46(12)	43(11)	45(13)	42(13)	44(12)	43(14)	36(12)
NO. (%) OF BLOOD DONORS	321	316	284	265			
By Exposure Groups							
#Low (AEI<3)	204(64)	244(77)	181(64)	203(77)			
#High (AEI≥3)	117(36)	72(23)	103(36)	62(23)			
By Exposure Levels							
#Low (AEI<1)	82(26)	99(31)	70(25)	75(28)			
#Intermed. (1-5)	196(61)	178(57)	172(60)	150(57)			
#High (AEI>5)	43(13)	39(12)	42(15)	40(15)			
NO. (%) OF FECAL DONORS	132	133	109	112			
By Exposure Groups							
#Low (AEI<3)	82(62)	106(80)	62(57)	84(75)			
#High (AEI≥3)	50(38)	27(20)	47(43)	28(25)			
By Exposure Levels							
#Low (AEI<1)	39(30)	37(28)	28(26)	31(28)			
#Intermed. (1-5)	72(55)	78(57)	62(57)	60(54)			
#High (AEI>5)	21(15)	18(15)	19(17)	21(18)			

and reservoir and the concentrations of the appropriate group of microorganisms in each wastewater source, varied across irrigation periods and are not taken into account in AEI. Thus, both RAEM and AEI would be required to assess the cumulative dose of a given microorganism group received by a given participant from the wastewater aerosol over a given irrigation period.

Most of the data analyses conducted involved a comparison of infection rates over an irrigation period among participants stratified by their degree of aerosol exposure. For these analyses, each participant was placed in the proper exposure category based on his AEI value during the relevant irrigation period. To perform each confirmatory statistical analysis, all participants were placed in either a ''high exposure'' or a ''low exposure'' group for the irrigation period. AEI=3.00 was the cutpoint used as the boundary between these two exposure groups. Suppose the value AEI=3.0 were obtained from EI=3.0 and XAEREM=0.0, for example. Then this value AEI=3.0 can be shown (see Section 4C) to be equivalent to staying on the Hancock farm for 24 hours per week throughout a spring irrigation period (or 16 hours per week throughout a summer irrigation period) without ever having extensive aerosol contacts downwind of an irrigating rig. To investigate a dose-response gradient during an irrigation period, incidence rates and risk ratios were determined for three aerosol exposure levels: low (AEI<1), intermediate (1≤AEI≤5) and high (AEI>5). The number of participants in the two exposure groups and the three exposure levels is presented in Table 43 for each irrigation period. It should be noted that many residents in the central portion of Wilson shifted from the high exposure group in the spring irrigation periods to the low exposure group in the summer irrigation periods because of differences in prevailing wind direction between the spring and summer irrigation periods. Most infections evaluated were determined from blood or fecal specimens. The breakdown of blood donors and fecal donors into the exposure groups and levels is also presented in Table 43.

Some analyses involved observation periods of a year or longer, i.e., 1982, 1983 or the entire irrigation period (1982 and 1983). A participant's aerosol exposure estimates AEI for each of these observation periods were calculated as weighted averages of his AEI values for the constituent irrigation seasons. Since most of the pathogens observed in infection episodes over these longer observation periods were enteroviruses, the weights for each irrigation season were calculated to be proportional to $W_c \cdot V$, the total number of enteroviruses sprayed from irrigating rigs during that irrigation season. The calculation procedure and resulting weights are presented in Table 44. For example, Table 44 indicates that the summer 1982 irrigation contributed 90.65% of the enteroviruses sprayed during 1982. Thus, a participant's AEI value for 1982 was calculated as $0.0935\ AEI_1 + 0.9065\ AEI_2$, where the subscripts 1 and 2 refer to spring 1982 and summer 1982, respectively. Table 43 also presents the distributions of AEI values thus obtained for 1982, 1983, and the entire irrigation period and the numbers of participants in the exposure groups and levels based on these values.

A few analyses involved the household as the unit of observation. A household aerosol exposure index, HAEI, defined as the maximum AEI among the household members during that irrigation period, was used as the exposure measure in these analyses. Since these analyses were conducted to take

TABLE 44. RELATIVE CONTRIBUTION OF IRRIGATION SEASONS TO TOTAL ENTEROVIRUSES SPRAYED FOR 1982, 1983 AND ENTIRE IRRIGATION PERIOD

	Irrigation season and irrigation dates			
	Spring 1982 2-16/4-30	Summer 1982 7-21/9-17	Spring 1983 2-15/4-30	Summer 1983 6-29/9-20
V, Volume of Wastewater Applied, cm				
From pipeline	5.83	6.91	0	0.20
From reservoir	-	3.87	14.85	14.99
W_c, Average Enterovirus Conc., pfu/mL				
Pipeline wastewater	0.0467	0.3732	0.0594	0.2692
Reservoir wastewater	-	0.0147	0.0018	0.0010
W_c x V				
Pipeline	0.2723	2.5788	0	0.0538
Reservoir	-	0.0569	0.0267	0.0150
Both	0.2723	2.6357	0.0267	0.0688
Relative Contribution to Total Sprayed (weight)[a]				
1982	9.35%	90.65%		
1983			28.3%	71.7%
Entire irrigation period (1982 + 1983)	9.1%	87.8%	0.9%	2.2%

[a] From W_c x V for both pipeline and reservoir irrigation.

within-household transmission of infection into account, the most highly exposed household member was considered to best represent the household's exposure.

AEI cannot be considered an ideal measure of the relative aerosol exposure of the participants within an irrigation season. Deficiencies include the lack of knowledge of the precise whereabouts of participants throughout the irrigation periods, the use of arbitrary weighting factors, and reliance on historical wind data rather than on actual on-site wind data from the irrigation periods.

Imprecise information regarding the specific wastewater aerosol exposure events experienced by each participant during 1982 was the primary limiting factor in the accuracy of AEI as a relative measure of aerosol exposure. The activity diary provided valuable information about participant habits during each irrigation period, especially regarding the amount of time spent at home, on the Hancock farm, and in Lubbock. However, in deference to respondent burden and privacy, the activity diary did not request detailed information about maximal exposure events. The degree to which the week of activity diary administration was representative of the entire irrigation period is unknown, although the activity diary weeks were selected to avoid holidays and school vacation breaks. The log of extensive wastewater contacts was introduced in 1983 to obtain much better data regarding maximal exposure events; this information was quantified in the indices of extensive exposures (XAEREM and XDIREM). XAEREM was incorporated as a component of AEI to obtain a better ranking of the relative exposure of the more highly exposed participants.

The sensitivity of the LISS results to alternative assignments of the arbitrary weighting factors employed in the AEI calculation has not been investigated, because of the extensive computations involved. However, other reasonable assignments are unlikely to significantly change the relative ranking of participants with regard to cumulative aerosol exposure.

Historical wind data was used to calculate the EI component of AEI for reasons of expendiency. This appears to have been justifiable in light of the greater uncertainty in AEI attributable to imprecise knowledge of participant exposure events in 1982. Wind roses for the actual irrigation periods were very similar to the historical wind roses except for the spring 1983 irrigation season. However, spring 1983 was the season of lowest aerosol exposure (Table 42), the fewest infection events occurred in the spring 1983 season (Tables 97-99), and there were no apparent associations with aerosol exposure in spring 1983 (Table 132). Hence, the use of the historical (rather than actual) wind data in calculating AEI should have had virtually no effect on the LISS findings.

To investigate the effects of these recognized deficiencies in AEI, the maximum aerosol exposure value of the household (HAEI) was plotted at the household location for each irrigation season. The resulting HAEI exposure isopleths appeared to be intuitively reasonable. In addition, the AEI values of household members were usually tightly clustered, except for individuals with occupational exposure to the wastewater. As an additional check, all of the AEI values calculated for every participant were reviewed for reasonableness by the health watch manager. The review revealed no significant classification error.

Additional Exposure Measures--

Other exposure measures were obtained to investigate alternative routes of wastewater irrigation exposure besides the wastewater aerosol. Each sentinel participant was asked to maintain a log of extensive wastewater contacts from February through September 1983. As part of the weekly illness report, the most extensive aerosol exposure and direct wastewater contact of the week and the estimated hours spent on the Hancock farm were also obtained for each household member. From these data, cumulative measures of extensive aerosol exposure (XAEREM) and direct wastewater contact (XDIREM) were calculated using the microenvironment method for each sentinel participant for both of the irrigation periods in 1983. The hours spent on the Hancock farm were also averaged as another exposure measure (FHRSEM). The calculation procedures were given in Section 4C.

The distributions of values of the additional exposure measures XAEREM, XDIREM and FHRSEM among all participants in the spring 1983 and summer 1983 irrigation periods are summarized by exposure levels in Table P-22 in Appendix P. Note that the percentage of participants with any extensive exposure was about 12% for extensive aerosol exposure, 6-8% for direct wastewater contact, and 19-24% for spending any time on the Hancock farm. The correlation among the exposure measures is indicated in Table P-23 in Appendix P. Note that the additional exposure measures are quite highly correlated with AEI ($0.365 \leq r \leq 0.610$) and very highly correlated with each other ($0.593 \leq r \leq 0.901$). Hence the other exposure measures were only employed in the data analysis when an association with AEI was indicated, in an attempt to identify the primary route of transmitting the infectious agent. The amount of time spent in Lubbock, TLUBOCK, was virtually uncorrelated with AEI (see Table P-23). Hence the time spent in Lubbock was incorporated in the exploratory data analyses to investigate direct contact with infected persons in Lubbock as an alternative hypothesis to exposure to Lubbock wastewater.

C. OTHER INVESTIGATED SOURCES OF MICROORGANISMS

Microorganism Levels on Flies

Baseline fly collection was attempted on several occasions, i.e., in August, September and October 1980. During August 4-5, 1980, baited fly traps were placed at locations adjacent to the Wilson effluent pond and at farmhouses on the Hancock property which would later be surrounded by wastewater sprinklers. Since collection attempts at these locations were unsuccessful, the effluent pond traps were moved 100 meters to a location adjacent to pig pens. Also, the traps placed near farmhouses were moved to locations which had livestock. On August 6-7, over 200 flies were collected at the pig pen locations and no flies were collected at any of the farmhouse locations. Viral analysis of the flies yielded no positive isolates. However, a variety of bacteria was recovered at densities ranging from very light to light (see Table 45). Bacterial levels may have been suppressed by the ether used to inactivate the flies.

A fly population developed following a period of rainfall in early September. A second fly collection was attempted on September 15 and 16 with traps located near the Wilson effluent pond, at two farms on the Hancock farm, and next to the school's trash can. No flies were collected during this attempt.

During a third attempt in October with traps at four locations, approximately 1,200 flies were collected (from October 15 to 17) near the pig pens adjacent to the Wilson sewage treatment facility, and approximately 65 flies were collected from October 20 to 22 in barns at farmhouses located near the reservoirs under construction on the Hancock farm. No viruses were recovered from either sample. Bacterial profiles are compared with the previous sample in Table 45. Staphylococcus aureus was present in moderate numbers in both samples collected in October. Additionally, Proteus vulgaris (in moderate numbers) and Salmonella arizonae were recovered from the sample collected at the pig pen. A variety of other organisms was isolated from the flies at low levels.

Fly collection during the irrigation period was attempted concurrently with the aerosol monitoring in summer 1982. These attempts were performed utilizing baited fly traps in the same manner as during the baseline year at locations adjacent to the reservoirs on the Hancock farm and the Wilson treatment facility. A fly collection attempt in August 1982 yielded insufficient flies for laboratory analysis. Surveillance for a significant increase in fly population was maintained until the first freeze, but conditions never warranted another attempt at fly collection.

Several fly collection efforts were also made during the summer 1983 irrigation. Fly samples were collected from July 19 to 22, 1983 at the intensive research plot on the Hancock farm, at Reservoir 1 on the Hancock farm, and next to the pig pen near the Wilson sewage treatment facility. These fly samples were scavenged by beetles while in the fly traps, then inadvertently kept cooled at 4°C for 3 weeks and held at room temperature for 24 hours prior to proper processing and analysis. A second attempt to collect flies in September 1983 was again unsuccessful because no flies were present.

The flies collected during July 19-22, 1983 yielded a bacterial profile that was similar to that observed with the flies collected during the baseline period. However, the levels of the respective organisms observed were generally higher in the flies collected during the irrigation period. The increased levels of organisms observed were undoubtedly affected by the problem in sample handling. The fly samples collected during the irrigation period were not analyzed for viruses due to the deteriorated state of the samples.

The fly data from the irrigation period is of questionable significance due to the problems in sample handling. However, the similarity in bacterial flora from baseline and irrigation periods suggests that the measurable flora was not altered by irrigation.

TABLE 45. BACTERIAL ISOLATES FROM FLIES

Sample source	No. of flies collected	Organism	Quantitation[a] of growth
Baseline			
Pig pen near Wilson effluent pond August 6-7, 1980	200[b]	Escherichia coli	L
		Hafnia alvei	L
		Staphylococcus aureus	L
		Klebsiella pneumoniae	VL
		Proteus mirabilis	VL
		Providencia stuartii	VL
		Staphylococcus epidermidis	VL
Pig pen near Wilson effluent pond October 15-17, 1980	1200	Proteus vulgaris	M
		Staphylococcus aureus	M
		Escherichia coli H_2S^+	L
		Fluorescent Pseudomonas gp.	VL
		Hafnia alvei	VL
		Klebsiella oxytoca	VL
		Salmonella arizonae	VL
Barn near Reservoir 3 Hancock farm October 20-22, 1980	65	Staphylococcus aureus	M
		Escherichia coli	L
		Fluorescent Pseudomonas gp.	L
		Klebsiella oxytoca	VL
		Serratia marcescens	VL
Irrigation			
Hancock farm (Rig 15) July 19-22, 1983	17[c]	Klebsiella pneumoniae	M
		Proteus mirabilis	M
		Serratia marcescens	M
Pig pen near Wilson effluent pond July 19-22, 1983	200[c]	Escherichia coli	M
		Providencia stuartii	M
		Serratia rubidaea	M
		Klebsiella pneumoniae	L
Hancock farm (Reservoir 1) July 19-22, 1983	44[c]	Serratia marcescens	H
		Klebsiella pneumoniae	M
		Proteus mirabilis	L
		Serratia odorifera	L

[a] Estimate of prevalence based on growth on primary culture plates (4 quadrants/plate):

H - heavy—growth in three or all quadrants
M - moderate--growth on first two quadrants
L - light—growth on first quadrant
VL - very light--one to ten colonies on plate

[b] Flies anesthesized with ether.

[c] Samples inadvertently held for 3 weeks prior to shipment for analysis.

The difficulty in collecting flies, both in the baseline period but especially after wastewater irrigation commenced, indicates that flies were not an important route of transmitting infectious agents at the study site, particularly during summer irrigation periods when the possibility of flies as an insect vector was most plausible. In marked contrast to the LISS experience, Echeverria et al. (1983) documented that the flies in a small rural village in northeastern Thailand frequently carried enteric pathogens and observed that size of the fly population and the incidence of diarrhea both increased in the hot dry season.

Microorganism Levels in Drinking Water

To assess contaminated drinking water as a potential source of the agents of infection episodes, samples of drinking water were obtained from a cross-section of rural households and from the Wilson water supply (see Figure 13). The results from analyses of the drinking water samples for total and fecal coliforms, fecal streptococci, and Salmonella are presented in Table 46.

Many of the drinking water wells on and adjacent to the Hancock farm showed evidence of microbial contamination after wastewater irrigation commenced. Each such well exhibited a high level of bacterial contamination before wastewater irrigation was initiated. Thus, there is no indication that wastewater irrigation operations were related to the contamination of drinking water wells on or near the Hancock farm.

Many of the rural household wells were either periodically or regularly contaminated, based on the data for the bacterial indicator organisms. These data indicate that viral and bacterial pathogens may also have been present quite frequently and sporadically in household drinking water wells throughout the rural study area. Therefore, microbial contamination of drinking water was investigated as a possible explanation for observed episodes of infection and illness, particularly as an alternative explanation when the pattern of occurrence suggested a possible association with wastewater irrigation (Section 5M).

The widespread occurrence of bacterially contaminated household drinking water supplies in the rural study area is consistent with the first national survey of rural water quality at the point of use conducted recently by Cornell University. Francis et al. (1984) found that 42% of households served by individual systems (single connection) had a total coliform density above 1 cfu/100 mL and that 1.6% of rural households had a fecal coliform density above 200 cfu/100 mL.

LCCIWR periodically notified each household of the test results on its drinking water well. Chlorination or other means of disinfection was recommended when warranted to eliminate bacterial contamination. No investigations were made to determine sources of well contamination or whether these sources resulted in any other personal exposure. Peak coliform concentrations in a well usually did not occur at the same time as peak fecal streptococci concentrations. This may be an indication that several different contamination sources were operating.

TABLE 46. MICROORGANISM DENSITIES IN DRINKING WATER IN THE STUDY AREA BY WELL LOCATION AND SAMPLING DATE

Household	Dates	Total coliform (colonies/100 mL)	Fecal coliform (colonies/100 mL)	Fecal streptococcus (colonies/100 mL)	Salmonella[a]	NO_3-N (mg/L)
On Hancock Farm						
118	10-14-81	>2000	14	0	+	6.17
	1-6-82	200	5	0[b]	–	0.37
	2-15-82	120	66	0	–	1.99
	6-22-82	1300	25	9	–	11.20
	11-4-82	47	0	0	–	2.23
	12-14-82	0	0	0	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	0	0	4	–	
	7-11-83	5	2	260	–	
	8-25-83	25	14	56	–	
	10-13-83	3	1	5	–	
120	11-5-81	570	20	0	–	0.74
	1-5-82	6000	59	0	–	0.94
	2-16-82	0	0	0	–	4.28
	6-16-82	60	0	330	–	5.71
	11-4-82	0	0	0	–	1.77
	12-14-82	0	0	0	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	1	–	
	5-31-83	0	0	0	–	
	7-11-83	0	0	0	–	
	8-25-83	0	0	3	–	
	10-13-83	50	21	122	–	
121	10-15-81	>2000	400	49	+	8.16
	1-4-82	20	2	0	+	1.90
	2-15-82	1	0	0	–	23.75
	6-16-82	100	50	3	–	25.48
	11-3-82	0	0	1	–	8.32
	12-14-82	0	0	1	–	
	3-28-83	0	0	10	–	
	5-3-83	6	0	0	–	
	5-31-83	0	0	0	–	
	7-11-83	0	0	23	–	
	8-25-83	0	0	0	–	
	10-12-83	37	37	1000	–	
123 (trailer)	7-12-83	7	1	4	–	
	8-24-83	1	0	0	–	
	10-12-83	60	0	0	–	
125	10-15-81	0	0	0	–	2.69
	1-4-82	15	0	0	–	0.62
	2-15-82	1700	28	9	–	2.35
	6-16-82	1200	NR	1	–	4.37
	11-3-82	0	0	0	–	4.53
	12-14-82	0	0	0	–	
	3-28-83	10	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	0	0	0	–	
	7-13-83	40	0	0	–	
	8-25-83	0	0	0	–	
	10-12-83	10	0	0	–	
131	10-14-81	140	30	3	–	0.45
	1-5-82	100	0	0	–	0.18
	2-15-82	0	0	0	–	1.36
	6-22-82	400	3	1	–	1.00

continued...

TABLE 46. (CONT'D)

Household	Dates	Total coliform (colonies/100 mL)	Fecal coliform (colonies/100 mL)	Fecal streptococcus (colonies/100 mL)	Salmonella[a]	NO_3-N (mg/L)
131 (Cont'd)	11-3-82	0	0	0	–	0.61
	12-14-82	0	0	0	–	
	3-28-83	0	0	0	–	
	5-3-83	1	0	1	–	
	5-31-83	0	0	0	–	
	7-13-83	10	0	0	–	
	8-25-83	2	0	0	–	
Within 400 m of Hancock Farm						
109	10-14-81	0	0	0	–	0.45
	1-5-82	0	0	0	–	0.10
	2-16-82	0	0	0	–	2.06
	6-16-82	0	0	0	–	0.75
	11-3-82	0	0	0	–	0.47
	12-14-82	0	0	0	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	0	0	0	–	
	7-11-83	3	0	22	–	
	8-24-83	0	0	0	–	
	10-12-83	0	0	6	–	
114	10-14-8	>2000	0	0	–	1.45
	1-6-82	800	20	0	–	0.40
	2-16-82	0	0	0	–	16.36
	6-22-82	0	0	0	–	1.81
	11-3-82	1	0	0	–	1.85
	12-14-82	0	0	0	–	
	3-28-83	3	0	0	–	
	5-3-83	0	0	4	–	
	5-31-83	0	0	0	–	
	7-13-83	0	0	0	–	
	8-24-83	0	0	0	–	
	10-13-83	0	0	0	–	
118	10-14-81	>2000	20	0	–	0.95
	1-6-82	0	0	0	–	0.15
	2-16-82	0	0	0	–	2.44
	6-22-82	300	30	4	–	1.07
	11-4-82	1	0	53	–	<0.01
	12-14-82	6	6	27	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	0	0	0	–	
	7-11-83	28	0	0	–	
	8-24-83	1	0	2	–	
	10-13-83	10	0	300	–	
122	12-15-82	9	0	1	–	
	3-28-83	1	1	15	–	
	5-3-83	1	0	73	–	
	5-31-83	90	3	91	–	
	7-11-83	9	0	0	–	
	8-25-83	22	0	3	–	
	10-13-83	10	2	101	–	
128	10-14-81	0	0	0	–	1.45
	1-6-82	0	0	0	–	0.26
	2-16-82	0	0	0	–	3.96
	6-16-82	0	0	0	–	1.70
	11-3-82	0	0	0	–	1.30
	12-14-82	0	0	0	–	
	3-28-83	0	0	0	–	

continued..

TABLE 46. (CONT'D)

Household	Dates	Total coliform (colonies/100 mL)	Fecal coliform (colonies/100 mL)	Fecal streptococcus (colonies/100 mL)	Salmonella[a]	NO_3-N (mg/L)
126 (Cont'd)	5-3-83	0	0	0	-	
	5-31-83	0	0	0	-	
	8-24-83	0	0	0	-	
	10-13-83	0	0	0	-	
320	10-31-81	0	0	0	-	9.47
	1-4-82	5	0	0	-	1.14
	2-16-82	0	0	0	-	17.28
	6-16-82	0	0	0	-	10.41
	11-4-82	0	0	0	-	4.27
	12-13-82	0	0	0	-	
	1-4-83	0	0	0	-	
	3-28-83	2	0	0	-	
	5-3-83	8	0	0	-	
	5-31-83	15	0	0	-	
	7-12-83	2	0	0	-	
	9-25-83	0	0	0	-	
	10-13-83	0	0	2	-	
City of Wilson						
298 (City Well 1)	10-14-81	0	0	0	-	5.42
	1-4-82	0	0	0	-	1.55
	2-15-82	0	0	0	-	7.15
	6-22-82	0	0	0	-	9.04
	11-4-82	0	0	0	-	<0.01
	12-13-82	0	0	0	-	
	8-24-83	0	0	0	-	
	10-13-83	0	0	0	-	
299 (Wilson treated water)	10-31-81	0	0	0	-	7.70
	1-4-82	0	0	0	-	1.21
	2-16-82	0	0	0	-	14.40
	6-22-82	0	0	0	-	7.55
	11-3-82	0	0	0	-	4.53
	12-13-82	0	0	0	-	
	3-28-83	0	0	0	-	
	5-3-83	0	0	0	-	
	5-31-83	0	0	0	-	
	7-11-83	0	0	0	-	
	8-24-83	0	0	0	-	
	10-12-83	0	0	0	-	
Beyond 400 m from Hancock Farm						
103	11-5-81	0	0	0	-	1.39
	1-5-82	0	0	0	-	10.02
	2-15-82	0	0	0	-	5.30
	6-22-82	0	0	0	-	9.18
	11-3-82	0	0	0	-	3.98
	12-13-82	0	0	0	-	
	3-28-83	0	0	0	-	
	5-3-83	0	0	0	-	
	5-31-83	0	0	0	-	
	7-11-83	0	0	0	-	
	8-24-83	0	0	0	-	
	10-12-83	0	0	0	-	
315	11-4-82	0	0	0	-	4.27
	12-15-82	2	0	0	-	
	3-28-83	0	0	0	-	
	5-3-83	0	0	0	-	
	5-31-83	0	0	0	-	
	7-13-83	6	0	0	-	
	8-24-83	0	0	0	-	
	10-13-83	0	0	0	-	

continued...

TABLE 46. (CONT'D)

Household	Dates	Total coliform (colonies/100 mL)	Fecal coliform (colonies/100 mL)	Fecal streptococcus (colonies/100 mL)	Salmonella[a]	NO_3-N (mg/L)
398	10-14-81	500	300	160	+	2.69
	1-4-82	80	0	0	–	0.40
	2-16-82	0	0	0	–	7.46
	6-16-82	19000	1000	60	–	3.74
	11-4-82	21	2	3	–	1.18
	12-13-82	100	3	0		
	3-28-83	200	110	13		
	5-3-83	0	0	0	–	
	5-31-83	21	2	0	–	
	7-12-83	0	0	0	–	
	8-25-83	0	0	0	–	
	10-14-83	10	1	90	–	
504	12-15-82	190	0	82	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	42	5	24	–	
	7-13-83	82	0	0	–	
	8-24-83	6	0	0	–	
	10-13-83	0	0	1	–	
531	11-4-82	0	0	0	–	1.90
	12-13-82	1	0	4	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	0	0	1	–	
	7-12-83	1	0	0	–	
	8-25-83	0	0	0	–	
	10-12-83	0	0	0	–	
540	12-15-82	0	0	0	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	1	1	0	–	
	7-13-83	0	0	0	–	
	8-24-83	0	0	0	–	
	10-12-83	0	0	0	–	
545	12-15-82	0	0	0	–	
	3-28-83	0	0	0	–	
	5-3-83	0	0	0	–	
	5-31-83	0	0	0	–	
	7-13-83	0	0	0	–	
	8-24-83	0	0	0	–	
	10-12-83	2	1	3	–	
546	12-15-82	≥8000	160	27	+	
	3-28-83	150	0	0	–	
	5-3-83	620	0	2	–	
	5-31-83	135	0	0	–	
	7-13-83	10	0	0	–	
	8-24-83	0	0	0	–	
	10-12-83	48	24	1	–	
555	12-15-82	1100	3	16	–	
	3-28-83	0	0	1	–	
	5-3-83	50	3	2500	–	
	5-31-83	10	5	1	–	
	7-13-83	2000	0	50	–	
	8-24-83	110	26	200	–	
	10-13-83	75	30	42	–	

[a] + Salmonella present (≥1 colony/100 mL)
– Salmonella not detected (<1 colony/100 mL)

[b] 0 no colonies detected with a detection limit of 1 colony/100 mL

The possible association with contaminated drinking water was investigated for each category of bacterial infection among all fecal donors (Section 5G). Contaminated drinking water was also investigated as an alternative explanation for each infection episode in which there was good or marginal evidence of a strong association with wastewater aerosol exposure (Table 133). Since the presence of bacterial indicator organisms is so widespread in rural drinking water supplies nationwide, a conservative definition of ''contaminated well water'' was employed in classifying each monitored rural household supply from the data in Table 46 for these analyses. When four to eight drinking water samples were analyzed during the period of observation for infections, the well was classified as contaminated if the average bacterial density per 100 mL of the samples exceeded 20 for total coliforms, 2 for fecal coliforms, or 5 for fecal streptococci, or if Salmonella was present in any sample. Since detection of bacterial contamination is less likely when fewer water samples are obtained, a less stringent criterion for contamination was used in this case. When only one to three drinking water samples were analyzed during the infection observation period, the well was considered to be contaminated if the average bacterial density per 100 mL exceeded 5 for total coliforms, 1 for fecal coliforms, or 2 for fecal streptococci, or if Salmonella was detected. With these criteria, slightly more than half of the monitored rural wells in Table 46 were classified as contaminated in most of the observation periods employed. The small number of participants whose household well was classified as contaminated but who only drank bottled water (i.e., never drank water from the faucet), were excluded from the contaminated drinking water group in the analyses of association with infection. Since the drinking water of 20 or fewer households was monitored during each period of observation, there often were insufficient data to detect an association of infections with contaminated drinking water, unless the infection rate was high or the association was very strong.

Monthly precipitation for the study area is presented in Table 47. There were 4 months of extremely heavy rainfall during the LISS. Rainfall exceeded the 40-year average by about 12 cm/mo in both May and June 1982 and by about 8 cm/mo in both August and October 1981. The extremely high densities of indicator bacteria in the rural drinking water (see Table 46) were most commonly observed in the October 1981 and June 1982 surveys (i.e., during months of excessive rainfall). The proportion of the rural household wells which were contaminated (by the criterion of the preceding paragraph) was found to be significantly associated with local rainfall in the sampling month ($r=0.576$, $p=0.025$). Some rural wells were reported to have been flooded by surface water runoff following heavy rainfall events in late May and June 1982. At some rural homes, the drinking water well was located close to the cesspool. Many of these cesspools were constructed improperly. This combination of circumstances appears to have contributed to the substantial and widespread contamination of the drinking water supplies of rural households, which was observed in the study area.

Although never documented through the water sample data, the possibility cannot be dismissed that the water supplied to households in Wilson was also contaminated sporadically. Prior to March 1983, the stored water obtained from six wells was only chlorinated periodically by hand prior

TABLE 47. PRECIPITATION (cm) BY MONTH IN THE STUDY AREA

	40-Year average	1980	1981	1982		1983	
Month	Lubbock airport	Lubbock airport	Lubbock airport	Hancock farm	Lubbock airport	Hancock farm	Lubbock airport
January	1.2	1.4	0.8	0.8	0.1	3.2	7.0
February	1.6	1.0	1.7	0.6	1.0	0.4	0.8
March	2.2	0.5	3.0	3.2	1.1	0.8	1.4
April	3.2	2.9	5.2	2.2	6.4	2.6	2.0
May	6.9	8.8	3.2	18.6	11.5	6.9	3.1
June	6.6	4.5	2.0	19.7	12.7	3.2	4.5
July	5.5	0.5	8.5	11.3	5.3	3.1	1.0
August	5.2	4.2	13.7	2.7	2.7	0	0.8
September	6.4	9.0	4.5	4.4	3.3	0.6	1.0
October	5.2	0.5	13.6	0.8	1.2		27.4
November	1.5	5.8	1.6	3.0	3.0		1.4
December	1.5	1.3	0.5	3.1	5.0		0.9
Annual	47.0	40.3	58.4	70.4	53.3		51.4

to distribution. Those households at the ends of branched 1-inch water lines in Wilson would have been most subject to the effects of bacterial contamination, since their drinking water tended to stagnate in the water lines. Any such effects were not investigated in the LISS.

Eating Food Prepared at Local Restaurants

Responses regarding patronage of the food preparation establishments in Wilson were obtained retrospectively in July 1984 for 117 routine fecal and illness specimen donors. Table 48 presents the distribution of responses by irrigation period for each ''restaurant.''

Since this was a small rural community, the majority of the respondents had no trouble with recall or knowledge of donor activity. Since all four establishments were located in the vicinity of the Wilson schools, most parents knew which ones their children did patronize both during the school year and in the summer when school was out. Patronage of the restaurants by the farm families was frequently determined by ''season.'' For example, some families were more likely to patronize the restaurants during planting season, some were more likely to patronize the restaurants when weeds were being sprayed (July-August), while others were more likely to patronize the restaurants during the harvest. In addition, the unusual weather conditions during the summer of 1982 made it easier for the respondents to recall instances when their patterns of restaurant patronage may have deviated.

It should be noted that restaurants A and B were the only ones which primarily served food, were open for business during both irrigation years, and were visited at least monthly by more than 10% of the surveyed donors. Most of the fecal and illness specimen donors reported the same frequency of eating food prepared at restaurants A and B during all four irrigation

TABLE 48. FREQUENCY DISTRIBUTIONS OF PATRONAGE OF MAJOR FOOD PREPARATION FACILITIES IN WILSON BY 117 FECAL AND ILLNESS SPECIMEN DONORS DURING IRRIGATION PERIODS

Frequency of patronage	Spring 1982	Summer 1982	Spring 1983	Summer 1983
Restaurant A				
never	69	63	71	65
<once/month	29	28	28	25
once/month to once/week	7	21	6	24
>once/week	12	5	12	3
Restaurant B				
never	71	77	71	75
<once/month	28	15	26	17
once/month to once/week	9	20	11	20
>once/week	9	5	9	5
Other facilities	Restaurant C		Restaurant D	
never	107	105	99	99
<once/month	4	3	11	8
once/month to once/week	0	8	0	8
>once/week	6	1	7	2

periods. However, there was a slightly greater tendency to patronize both restaurants at least monthly during the summer (i.e., June-August) when school was out. The demographic characteristics of patrons are compared to those of nonpatrons below, based on patronage during summer 1982. Very similar patronage patterns were obtained for summer 1983, but the summer 1982 patterns are reported below because this was the season of initial interest when the restaurant patronage survey was designed.

Restaurant A--

Twenty-two percent of the 117 illness and fecal specimen donors who were surveyed reported eating food prepared at restaurant A at least once a month. Twenty-four percent of the donors reported eating food prepared by restaurant A less frequently than once a month. Fifty-three percent of the donors reported that they never ate food prepared by restaurant A.

The restaurant A patrons differed from the nonpatrons for six of the seven demographic variables examined. Restaurant A patrons tended to be younger that nonpatrons ($p<0.001$), were more likely to be male than female ($p=0.077$), were likely to live in households where the head of household's 1979 income was reported to be in the $10,000-19,999 range ($p=0.064$), and were more likely to live in Wilson than in a rural area ($p=0.030$). Hispanic donors were more likely to patronize the restaurant than caucasian donors ($p=0.032$). There were no differences found between patrons and nonpatrons for the head of household education variable. The donors in the three exposure levels differed in their frequency of restaurant A patronage ($p<0.001$). Seventy-nine percent of the respondents in the high exposure level reported eating food prepared by restaurant A more frequently than once a month. Only 6% in the low exposure level reported patronizing restaurant A more than once a month.

Restaurant B--

Twenty-two percent of the donors reported that they ate food prepared at restaurant B more frequently than once a month. Thirteen percent of the donors ate food prepared by restaurant B less frequently than once a month. Sixty-six percent of the donors reported that they never patronized the restaurant.

Patrons of restaurant B differed from nonpatrons in four of the seven demographic characteristics examined. The restaurant patrons were found to be younger than nonpatrons ($p<0.001$); patrons lived in Wilson more frequently than in the rural area ($p=0.002$); hispanic donors were more likely to patronize the restaurant than were caucasian donors ($p<0.001$); and donors from households where the head of household's 1979 income was reported to be in the $10,000-19,999 range were more likely to patronize the restaurant than were donors from households with higher and lower incomes ($p=0.025$). Patrons and nonpatrons did not differ for the variables of sex, head of household education, and exposure level.

Discussion--

There are common factors which were associated with patronage of restaurants A and B. Geographic location of the household in relation to the restaurant was important in determining restaurant patronage. Those living in the proximity of Wilson found the restaurants more convenient than did those donors who lived on the outside edges of the study area. Household income was also important. Donors from households with low incomes could not afford to patronize the restaurants, while donors from high income households were more likely to travel to a larger community for a meal. Age was important in determining which of the middle income donors (who lived in or near Wilson) actually patronized the restaurant on a frequent basis. Children ages 6-17 and adults ages 18-44 were more likely to buy food from these restaurants. Some children, most of whom were hispanic residents of Wilson, reported frequenting the establishments on a routine basis. The adults reported buying food from the restaurants only when they were ''too tired to cook'' or ''in a hurry.''

Patronage of restaurant A was much greater among surveyed donors with a high level of wastewater aerosol exposure. Thus, any health effects of wastewater aerosol exposure may be confounded with any health effects of eating food prepared by restaurant A in the LISS population. To allow valid interpretation, it is necessary to investigate eating food prepared by restaurant A as an alternative explanation to any apparent association of infections with aerosol exposure. This exploratory analysis was performed by logistic regression for the surveyed donors and is presented in Section 5L.

D. DESCRIPTION OF STUDY POPULATION

Questionnaire Data

Tables P-24 to P-30 of Appendix P report information derived from interviews with members of the 163 participating households. The questionnaires used in these interviews were designed by the University of Illinois School of Public Health. Interviews were administered in respondents' homes in 1980 and by telephone in 1982 and 1983. Copies of these questionnaires can be found in Appendices B, C and D. A detailed description of the interview procedure is presented in Section 4B. Only responses from individuals or households which actually participated in the study (i.e., provided health diary information, blood samples or fecal specimens) were tabulated. Every effort was made to resolve inconsistencies and to correct omissions. However, four individuals are included in Tables P-25 and P-27 to P-29 in Appendix P who were considered to be nonparticipants elsewhere in this report, since they only provided an initial blood sample. The heading NR was used as an abbreviation for ''not recorded'' for the few cases where the household withdrew from the study before the missing information could be obtained. With the exception of the farm information, the material summarized in the tables is discussed in greater detail in subsequent portions of this report.

Tables P-24 and P-25 in Appendix P present information concerning household and individual characteristics of the study population based on responses to the initial (May 1980) and final (October 1983) questionnaires. Tables P-26 and P-27 in Appendix P present crosstabulations of the overall exposure levels (based on combined 1982-1983 aerosol exposure indices) with selected household and individual variables of interest in the study. These crosstabulations are used only to provide the reader with an understanding of the general demographic patterns observed in the study. Since irrigation patterns varied between the spring and summer seasons as well as between 1982 and 1983, the degree of exposure of individuals in the study population also varied between time intervals. Therefore, the patterns observed in Tables P-26 and P-27 only summarize general trends. Table P-28 contains crosstabulations of selected demographic variables which allow the population to be characterized by age, sex, race, and household location. Table P-29 summarizes the health history information obtained from participants. Table P-30 summarizes crop and livestock information provided by participating farm households. The farm data provide indications that farming activity in the community declined substantially during the course of the study.

A capsule description of the study population based on participants remaining with the study until its completion is presented based on Tables P-24, P-25 and P-28 and other sources. The racial composition of the study population was 72% caucasian and 28% hispanic. Males and females each comprised about half of the participants in each age group. The size of households was 22% single member, 37% two member, and 17% with five or more members.

Farming was the primary occupation and 58% of the heads of household had completed high school. All participants lived in single family dwellings, of which 39% had evaporative coolers and 44% had refrigerated air conditioning.

Approximately 95% of the study population visited Lubbock at least once per month, with a median of about 16 hours per month spent there.

The study population included 17 tenant farmers and workers who had regular direct contact with wastewater and heavy aerosol exposure on the Hancock farm. An additional 21 participants in 10 households lived within 200 m of the spray irrigation. Eight homes of 19 participants were located within 50 m of a sprinkler irrigation circle and many of them thereby received substantial aerosol exposure.

Population Demographics

Crosstabulations of specific demographic variables obtained from the three questionnaires (administered in 1980, 1982, and 1983) were generated to determine if:

- self-selection altered the characteristics of the LISS population during the course of the study;
- the major subgroups in the population were similar in terms of socioeconomic status, age, geographic distribution, family size and other demographic characteristics;
- the various donor groups differed significantly from the overall study population with regard to demographic characteristics;
- the two exposure groups and the three exposure levels were balanced with respect to demographic characteristics.

A description of each variable as well as the value categories for each of the participant characteristics is contained in Table 49. Variables of interest included personal information such as age, race, sex, socioeconomic status, smoking habits, and history of chronic illness. Environmental variables of interest included household size, presence (or absence) of children in the household, source of drinking water, air conditioner use, and household location. Family income and the occupation and education level of the head of household were used as indicators of socioeconomic status for all household members. However, since 44% of the study participants lived in households headed by farmers, and since annual farm income was found to be unstable during the course of the study, the head of household's education was considered to be the most reliable of the three socioeconomic indicators.

The appropriate Cochran-Mantel-Haenszel statistics were used to generate the ''p values'' for all crosstabulations. The ''p values'' are listed (in Tables 50, 51 and P-31 to P-44) only when equal to 0.10 or less. Each p-value below 0.05 was interpreted to indicate a significant difference between the subpopulations being compared. The categories of household size, income, and education were collapsed to meet the criterion that no more than 20% of the cells had an expected frequency of 5 or less. In cases where the same question had been administered in two or more questionnaires (e.g., household size, smoking, and bottled water consumption), the most recent response from each participant was used.

TABLE 49. VARIABLES USED IN DEMOGRAPHIC ANALYSIS

Household variables

ACOND: Do you have air conditiong in home
1 Yes
0 No

ACSYS: Air conditioning system
0 None
1 Refrigeration
2 Evaporative cooler
3 None

DWATER-B: Drinking water supply (modified to include bottled water consumers)
0 Bottled water
1 Private well
2 Public supply

GHSIZE: Grouped household size
1 1 person
2 2-4 people
3 >5 people

GINCOME: Grouped income
1 <5,000
2 5000 to 9999
3 10000 to 19999
4 20000 to 29999
5 >30000

HCHILD: Age of youngest child in household
1 No children
2 Child 6-17
3 Child ≤ 5

HOHEDGR: Education category of head of household
1 0-8
2 9-11
3 12
4 Some college (13-15)
5 College grad (16-18)
(Categories 4 and 5 combined for some tests.)

HOHOCC: Head of household occupation group
1 Professional or manager
2 Farmer
3 Other

LOCATE: Dwelling location
1 Rural
2 Wilson

Individual variables

ABDOM: Any abdominal conditions?
0 No
1 Yes
8 Don't know

AGEGRP: Age group (as of June 30, 1982)
1 0-5
2 6-17
3 18-44
4 45-64
5 65+

BOTTLED: Drinks bottled water regularly
0 No
1 Yes

CHRONIC: History of any chronic illness
0 No
1 Yes

CONTACT: Contacts per week with 10+ people
1 Less than once
2 1 to 5
3 6 to 10
4 11 to 15
5 More than 15

HEART: Any heart conditions
0 No
1 Yes
8 Don't know

OTHERO: Any other chronic conditions
1 Yes
0 No

RESP: Any respiratory illness
0 No
1 Yes
8 Don't know

SEX: Sex
1 Male
2 Female

SMOKE3: Smoke cigarettes regularly in 1983 (or most recent questionnaire)
0 No
1 Yes

continued...

TABLE 49. (CONT'D)

Household variables	Individual variables
RACE: Race of respondent	TCHEW: Chew tobacco regularly
1 Caucasian	0 No
4 Hispanic	1 Yes
SENTINL: Sentinel family status for 1983	WCONSM: Tapwater consumed vs. others your age
1 Yes (sentinel HH)	1 Less than average
0 No	2 Average
ZONE: Household location	3 More than average
1 Rural 0 to 0.5 mile	
2 Wilson 0 to 0.5 mile	
3 Rural 0.5 to 1 mile	
4 Wilson 0.5 to 1 mile	
5 Rural 1 to 2 miles	
6 Workers >2 miles	

TABLE 50. COMPARISON OF CHARACTERISTICS: STUDY PARTICIPANTS VS. NONPARTICIPANTS

Variable	n	P value	Comment
ACOND	577	0.045	''none'' associated with nonparticipation
ACSYS	577		
ABDOM	575	0.045	''yes'' associated with participation
AGEGRP	577	0.014	''65+'' associated with participation
BOTTLED3	575		
CHRONIC	568	0.006	''yes'' associated with participation
DWATER-B	577	0.028	''bottled water'' associated with participation
GHSIZE	578		
GINCOME	577		
HCHILD	578	0.038	households with kids ages '6-17' assoc with nonparticipation
HEART	574		
HOHEDGR	574	0.025	''some college'' associated with participation
HOHOCC	577	0.012	''prof or manage'' associated with participation
LOCATE	578	0.042	''rural'' associated with nonparticipation
OTHERO	577	0.06	''yes'' associated with participation
RACE	578	0.001	''hispanic'' associated with nonparticipation
RESP	577	0.017	''yes'' associated with participation
SEX	578		
SMOKE	577		
ZONE	578	<0.001	zones 3-5 associated with nonparticipation

TABLE 51. COMPARISON OF CHARACTERISTICS: PARTICIPANTS WHO REMAINED IN THE STUDY VS. PARTICIPANTS WHO DROPPED OUT

Variable	n	p value	Comment
ACOND	475	0.013	higher proportion of ''none'' dropped out
ACSYS	339	<0.001	higher proportion of ''none'' dropped out
ABDOM	477		
AGEGRP	477	0.003	higher proportion of age 45+ continued participation
BOTTLED3	478		
CHRONIC	478	0.001	higher proportion of ''yes'' continued participation
DWATER-B	478		
GHSIZE	468		
GINCOME	468		
HCHILD	478	0.012	higher proportion of ''no children'' continued participation
HEART	477		
HOHEDGR	474	0.013	college education associated with continued participation
HOHOCC	475	0.003	higher proportion of ''other'' dropped out
LOCATE	478		
OTHERO	477	0.025	higher proportion of ''yes'' continued participation
RACE	478	0.002	higher proportion of ''hispanic'' dropped out
RESP	477		
SEX	478		
ZONE	478	0.034	higher proportion of zones 2 and 3 dropped out

Effect of Self-selection on LISS Population Characteristics--

Analysis of the questionnaire data indicates that although great efforts were taken during recruitment to select households which were representative of the study area, the process of self-selection resulted in some significant demographic changes during the course of the study. In fact, the characteristics of the population changed between the time that the initial households were recruited (May 1980), and the time that the first blood samples and illness diaries were collected (June 1980). One hundred ninety-six households with 578 members were initially recruited into the study. Thirty-three of those households (with 100 members) never actually participated in the study. Comparison of the nonparticipating households to the 163 participating households (with 482 members) in Table 50 indicates that the two populations were significantly different for 12 of the 20 variables examined. It can be seen in Table 50 that residents living more that 1/2 mile from the Hancock farm (sampling zones 3-5), hispanics, and families with children ages 6-17 were more likely to refuse to participate in the study. People with a history of chronic illnesses, members of households with high socioeconomic status, and members of families with children ages 0-5 were more likely to initially participate in the study.

Sixty percent of the study participants (55% of the households) remained with the study until its conclusion in October 1983. Twenty-four percent of the participants dropped from the study prior to the onset of irrigation; another 12% dropped during the irrigation period. Comparison of the participants who remained in the study until October 1983 to the participants who dropped out (Table 51) indicates that the two populations differed significantly for 10 of the 19 variables examined. Hispanics and participants under the age of 45 were more likely to drop out of the study before its conclusion. Participants living in high economic status households, participants with a history of chronic illness, and participants living in households with no children were more likely to stay with the study until its conclusion.

As a result of self-selection, the 288 participants who remained in the study until its conclusion in 1983 probably were not representative of the community surrounding the Hancock farm. The study participants were somewhat older, had a higher socioeconomic status, reported more chronic illnesses, and had less exposure to small children than did the members of the general community. Since their socioeconomic status was higher and their exposure to small children in the household was reduced, the study population's risk of infection (by agents of concern to the LISS) was probably somehwat lower than the infection risk of the general population. Due to the increased age and the higher rate of chronic illness in the study population, it might be expected that symptoms of illness (resulting from infections by agents which were circulating through the community) would be more severe in the study population than in the overall population.

Characteristics of Subpopulations--

Tables P-31 to P-33 of Appendix P list the results of the crosstabulations used to determine if there were demographic differences between subpopulations stratified on three key characteristics: race (caucasian vs. hispanic), sampling zone, and residence location (Wilson vs. rural). These analyses were performed in order to identify the presence of confounding variables which could affect the interpretation of results of other statistical tests.

Hispanics and caucasians differed significantly for every variable tested except sex, head of household occupation, smoking, and use of bottled water (Table P-31). Hispanic participants lived in households with more family members, were generally younger and reported a lower socioeconomic status than caucasian participants. Forty percent of hispanic participants were under the age of 18; only 25% of caucasian participants were in the same age group. Only 4% of the hispanics were age 65 or older; 17% of caucasians were age 65 or older. One percent of hispanic participants and 10% of caucasian participants reported living in single member households. In contrast, 23% of caucasian participants and 68% of hispanic participants reported that they lived in households with five or more members. Sixty-two percent of caucasian participants had experienced one or more chronic conditions. Only 28% of hispanic participants reported experiencing any chronic conditions. The difference in reporting of chronic conditions is not surprising in view of the fact that almost half of the hispanic participants were under the age of 18 and had no opportunity to develop many of the chronic conditions which are associated with aging.

Wilson participants and rural participants were found to be significantly different for 8 of the 20 variables examined (Table P-32). The majority of these differences can be attributed to the fact that 90% of the hispanic participants lived in Wilson. In addition, 60% of the single member households were also located in Wilson. The majority of participants living in single member households were over the age of 65. The clustering of the low income hispanic population with the elderly population on a fixed income caused the Wilson participants to have a significantly lower household income than the rural residents.

Sampling zone residents were found to differ significantly for 10 of the 20 variables examined (Table P-33). Zone 1 reported a higher socioeconomic status, fewer households with children, a higher proportion of farmers as head of household, and a higher proportion of chronic GI illnesses. Zone 3 had the highest proportion of participants who drank bottled water, the highest proportion of chronic illnesses, and the lowest proportion of smokers. Zone 4 participants drank less bottled water, reported the fewest chronic illnesses, and had a higher proportion of both single member and five-or-more-member households.

The presence of significant differences between subpopulations, especially the differences observed between races, is of some concern in this study. If hispanic households had been evenly distributed throughout the study area, the differences between the two races would not have impacted the study. Since the majority of hispanic households were located in Wilson, the geographic distribution of the ''susceptible'' population was affected. The lower standard of living, larger household sizes, and more frequent contact with children all increase the hispanic participants' risk of exposure to infectious agents. Therefore, the risk of infection (caused by the agents of concern in this study) was theoretically greater in the Wilson area than in the surrounding rural area. The presence of a higher standard of living in Zone 1 coupled with the absence of children in over half of Zone 1 households suggests that the risk of infection was comparatively small for residents and neighbors of the Hancock Farm. Based on demographic differences (and on the assumption of no effect of wastewater aerosol), the acute illness rate was expected to be greater in the Wilson area than in the vicinity of the Hancock farm. It was also expected that the differences (in illness and infection rates) between Wilson and the surrounding area would decrease as the process of self-selection caused the Wilson and Hancock farm residents to become demographically more similar as the study progressed (Table 51).

Characteristics of Donor Groups--

Four hundred thirty-five (91%) of the 478 participants provided at least one blood specimen during the course of the study. Thirty-three percent of the participants provided all eight of the requested bloods, 43% of the participants provided four to seven of the requested bloods. Twenty-four percent of the participants provided one to three bloods; this group includes children who were born during the course of the study and participants who dropped out of the study prior to the onset of irrigation. Comparison of the three groups of blood donors (Table P-34) reveals that these groups differed significantly for 13 of the 20 variables examined. Since the grouping of blood donors is similar to the grouping used to compare participants who remained in the study to those who dropped out (Table 51), significant differences in age, race, chronic illness history, and socioeconomic status were expected. Blood donor groups differed for two additional characteristics, drinking water source and household location. Wilson residents and participants who drank bottled water were more responsive to requests for blood samples. In terms of transportation and convenience, it was easier for Wilson residents to provide blood samples. Rural residents who lived on unpaved roads had more difficulty providing the samples, especially in June and December 1982, when inclement weather frequently caused roads to be impassable.

Table P-35 of Appendix P compares ''sentinel'' participants to the remainder of the study participants. Sentinel participants were the only study participants who were asked to continue to provide illness information between October 1982 and October 1983. All Zone 1 families and all study participants with wastewater contact were automatically included in the sentinel group. The remainder of the sentinel families were selected on the basis of three criteria: their willingness to continue to participate in the study, a history of chronic illness, and demographic similarity to the households in Zone 1. Since Zone 1 families differed demographically from the rest of the study population (Table P-33), all but one of the significant differences observed between the sentinel family members and the participant population were expected. The unexpected difference, less smoking in the sentinel participants than in the overall population, did not appear to be associated with any of the other demographic variables except sampling zone (Table P-33). There were more smokers located in Zone 2; however, Zone 2 was adequately represented in the sentinel population.

Tables P-36 to P-38 in Appendix P list the results of analyses which compared fecal donors from each irrigation season to the remainder of the participant population. Due to the small number of participants (primarily children) who provided specimens during 1980-1981, no comparison of donors to nondonors could be made for that period of time. There were no significant differences between fecal donors and nondonors in the spring of 1982. There was a higher proportion of fecal donors from low income households in the summer of 1982. There were also significantly fewer donors from households with children ages 6-17 during that same period of time. There were significantly more fecal donors with chronic conditions and fecal donors living in single member households during both irrigation periods in 1983. Cigarette smokers and hispanics were less likely to be donors during 1983.

The gradual increase in demographic differences between fecal donors and nondonors in 1982 and 1983 can be explained by the fact that the rules for donating the specimens were changed between 1982 and 1983. The number of specimens accepted from each household was limited to two in 1983 to reduce costs; there was no similar restriction in 1982. Therefore, many children (especially children from hispanic households) who donated specimens in 1980-1982 were excluded in 1983. Also, the potential fecal donors were randomly selected as donors in January 1982. Therefore, differences between donors and nondonors were expected to be minimal at that time. As the study progressed, it appears that the process of self-selection became more influential in determining who would donate specimens, and the demographic differences increased accordingly.

Exposure Categories Based on Aerosol Exposure Indices--

Tables P-39 to P-44 in Appendix P list the demographic differences observed between the two exposure groups and the three exposure levels for each of the four irrigation periods, for 1982 and for 1983. Comparison of the characteristics of the high and low exposure subgroups of blood donors and fecal donors is provided as the preliminary statistical analysis in Section 5L.

A quick review of the information in Tables P-39 to P-44 reveals significant differences between exposure levels during all periods for the variables DWATER, LOCATE, and ZONE. These differences can be explained by the fact that the majority of participants with medium exposure to wastewater aerosols lived in Wilson. There was also a significant difference between both exposure groups and exposure levels for type of air conditioning system in use during all periods of interest. The high exposure group and high exposure level consistently used more evaporative cooler units for air conditioning than did the remainder of the study population. There were no differences between exposure groups or between exposure levels for the variables age, bottled water consumption, and history of chronic illness.

Overall, there were more significant differences between exposure levels than between exposure groups for the majority of the variables. In addition, the variables associated with significant differences between exposure levels (income, occupation, household size) were the same variables for which significant differences were found when comparing Wilson residents to rural residents. However, since portions of the Wilson population were incorporated into both the high and low exposure groups, fewer significant differences were observed between exposure groups.

The presence of significant differences by exposure level, exposure group, and subpopulations (i.e., race) necessitated the exploratory statistical analysis of infection episodes by logistic regression to investigate their effects and to control the association of infection status with aerosol exposure for their effects. This analysis is presented in Section 5L.

Samples Provided by Study Population During the Health Watch

Table 52 lists the number of samples obtained from the various health watch activities by data collection period (DCP) during the course of the study. This table provides an overview of the scope and extent of the health watch of the study population which the LISS maintained. Some of the LISS results are subsequently reported by DCP. The first two columns of Table 52 give the correspondence between DCP and calendar date for the interested reader.

E. PATTERNS IN SELF-REPORTED ILLNESS

Study participants were contacted on a regular basis for illness information during the study period. All participating households were asked to keep a written illness diary in 1980; field representatives collected the illness information by phone in 1981-1983. All households were contacted for diaries in 1980-October 1982. Only sentinel families were contacted for illness information after October 1982. The written diaries were collected at 2-week (data collection period, DCP) intervals in 1980. The households were contacted by phone on a weekly basis in 1981-1983, and the weekly information was combined and coded for each DCP. Household members were asked to report all acute and chronic illness conditions which occurred during the time interval of interest. Participants were also asked to report the number of days of illness that they experienced as well as the number of days that they spent away from the study area.

For purposes of summarization, illnesses have been categorized into five groups: total acute illness, respiratory illness, gastrointestinal illness, other acute illness, and chronic conditions. Cases of trauma and elective surgeries were recorded, but were not used in the data analysis. An illness with both respiratory and gastrointestinal symptoms was treated as being two distinct illnesses. Respiratory, gastrointestinal, and other acute illnesses were included in the category ''total acute illness.'' ''Other acute illness'' included all acute illnesses which were neither respiratory nor gastrointestinal in nature. These illnesses included but were not limited to eye and ear infections, childhood diseases, headaches without accompanying symptoms, fevers of unknown origin, genitourinary infections, and various skin conditions. Newly developed chronic conditions and flare-ups of existing chronic conditions (such as arthritis) were recorded whenever reported. However, reporting of chronic conditions in this study was found to be quite erratic.

TABLE 52. NUMBER OF SAMPLES COLLECTED FROM HEALTH WATCH ACTIVITIES

Data collection period	Starting date	Households interviewed	Participant interview data	Health diaries	Polio immuni-zations	Blood specimens	Skin tests	Routine fecal specimens	Illness specimens	Activity diaries	Major irrigation periods
1980											
001	Jan 1										
002	Jan 13										
003	Jan 27										
004	Feb 10										
005	Feb 24										
006	Mar 9										
007	Mar 23										
008	Apr 6										
009	Apr 20										
010	May 4										
011	May 18	197[a]	580[a]								
012	Jun 1					318	265				
013	Jun 15										
014	Jun 29			348							
015	Jul 13			366				22			
016	Jul 27			364							
017	Aug 10			351				36			
018	Aug 24			342							
019	Sep 7			331				47	3		
020	Sep 21			336							
021	Oct 5										
022	Oct 19										
023	Nov 2										
024	Nov 16										
025	Nov 30					363	33				
026	Dec 14										
1981											
101	Dec 28										
102	Jan 11										
103	Jan 25										
104	Feb 8										
105	Feb 22										
106	Mar 8										
107	Mar 22										
108	Apr 5			402	49			24			
109	Apr 19			409							
110	May 3			405	105			11			
111	May 17			386							
112	May 31			375	4			45			
113	Jun 14			396	76	287	187				
114	Jun 28			401	1			30			
115	Jul 12			406	1						

continued...

TABLE 52. (CONT'D)

Data collection period	Starting date	Households interviewed	Participant interview data	Health diaries	Polio immuni-zations	Blood specimens	Skin tests	Routine fecal specimens	Illness specimens	Activity diaries	Major irrigation periods
116	Jul 26				22						
117	Aug 9			407				11			
118	Aug 23			405	6			34			
119	Sep 6			413	3			8			
120	Sep 20										
121	Oct 4										
122	Oct 18										
123	Nov 1										
124	Nov 15										
125	Nov 29										
126	Dec 13										
1982											
201	Jan 3			350	41	330		107	2		
202	Jan 17	129	365	381	2				4		
203	Jan 31			387	3				5		
204	Feb 14			386	3						Feb 16-
205	Feb 28			387	10			127			X
206	Mar 14			388	3				1	194	X
207	Mar 28			386	7			127	7		X
208	Apr 11			387						156	X
209	Apr 25			389	9						-Apr 30
210	May 9			389	1				2		
211	May 23			387	6						
212	Jun 6			370		310		124	5		
213	Jun 20			373	3				4		
214	Jul 4			367	2						
215	Jul 18			367	1				6		Jul 21-
216	Aug 1			359				119	7	261	X
217	Aug 15			354					1		X
218	Aug 29			352					3		X
219	Sep 12			351				121	16		-Sep 17
220	Sep 26			360	1				15		
221	Oct 10			357					8		
222	Oct 24			175					4		
223	Nov 7			175					5		
224	Nov 21			175					11	332	
225	Dec 5			180	10	268	245		6		
226	Dec 19								2		
1983											
301	Jan 2			181					12		
302	Jan 16			181					5		
303	Jan 30			181	1		15	100	12		

continued...

TABLE 52. (CONT'D)

Data collection period	Starting date	Households interviewed	Participant interview data	Health diaries	Polio immuni-zations	Blood specimens	Skin tests	Routine fecal specimens	Illness specimens	Activity diaries	Major irrigation periods
304	Feb 13			181	1				7		Feb 15–
305	Feb 27			181					4		X
306	Mar 13			181						10	X
307	Mar 27			181	5						X
308	Apr 10			182				109	2	309	X
309	Apr 24			183	5				4		–Apr 30
310	May 8			183					2		
311	May 22			183	5				17		
312	Jun 5			176	1	273		102	7		
313	Jun 19			175					3		Jun 29–
314	Jul 3			175						317	X
315	Jul 17			168				105	2		X
316	Jul 31			168						17	X
317	Aug 14			165				101	2		X
318	Aug 28			156					2		X
319	Sep 11			159							–Sep 20
320	Sep 25	107	306	161		267	202				
321	Oct 9										
322	Oct 23										
323	Nov 6										
324	Nov 20										
325	Dec 4										
326	Dec 18										

a The household head or spouse was interviewed upon recruitment regarding all household members. One–hundred fifty six household interviews of 430 members occurred in DCP 011 or 012, but replacement households and new family members were recruited into the study until DCP 212. Thirty four of the 197 interviewed households (102 of 580 members) which were recruited in DCP 011 never actually participated in the study.

TABLE 53. MONTHLY INTERVALS FOR SELF-REPORTED ILLNESS DATA BY DATE AND DCP

Month	DCPs	Dates
Jul 1980	014-015	Jun 29-Jul 26
Aug 1980	016-017	Jul 27-Aug 23
Sep 1980	018-020	Aug 24-Oct 4
Apr 1981	108-109	Apr 5-May 2
May 1981	110-111	May 3-May 30
Jun 1981	112-113	May 31-Jun 27
Jul 1981	114-115	Jun 28-Jul 25
Aug 1981	117-118	Aug 9-Sep 5
Sep 1981	119	Sep 6-Sep 19
Jan 1982	201-202	Jan 3-Jan 30
Feb 1982	203-204	Jan 31-Feb 27
Mar 1982	205-206	Feb 28-Mar 27
Apr 1982	207-209	Mar 28-May 8
May 1982	210-211	May 9-Jun 5
Jun 1982	212-213	Jun 6-Jul 3
Jul 1982	214-215	Jul 4-Jul 31
Aug 1982	216-217	Aug 1-Aug 28
Sep 1982	218-219	Aug 29-Sep 25
Oct 1982	220-222	Sep 26-Nov 6
Nov 1982	223-224	Nov 7-Dec 4
Dec 1982	225	Dec 5-Dec 18
Jan 1983	301-302	Jan 2-Jan 29
Feb 1983	303-304	Jan 30-Feb 26
Mar 1983	305-306	Feb 27-Mar 26
Apr 1983	307-309	Mar 27-May 7
May 1983	310-311	May 8-Jun 4
Jun 1983	312-313	Jun 5-Jul 2
Jul 1983	314-315	Jul 3-Jul 30
Aug 1983	316-317	Jul 31-Aug 27
Sep 1983	318-320	Aug 28-Oct 8

Two measures of illness were employed to characterize the self-reported illness. Incidence density, defined as the number of new illnesses per 1000 person-days of observation, was used to measure the occurrence of new illness in the population. Prevalence density, defined as the number of person-days of illness per 1000 person-days of observation, is a period prevalence measurement which was used to characterize the burden or duration of the illnesses which were observed during a given period of time. These rates were calculated for both the two exposure groups and the three exposure levels (based on AEI calculations) for ''monthly'' intervals of time. The AEI values from the spring 1982 irrigation period were used to determine exposure groups and levels for the illness data from July 1980 through May 1982. The summer 1982 AEI values determined exposure groupings for the June through December 1982 illness data. For 1983, the correspondence used was: spring 1983 AEI for January-May 1983 and summer 1983 AEI for June-September 1983. Since all data were collected on a 2-week basis, the DCPs did not always correspond with the exact beginning and ending of each of the months. Table 53 lists the DCPs which correspond with each of the months used to present the self-reported illness information.

It should be noted that all of the self-reported illness information, especially the baseline information, should be interpreted with extreme caution. In addition to the normal problems and biases that are encountered with self-reported data, the methodology for collecting this information was revised several times during the course of the study in order to improve the consistency, reliability, and completeness of the information. Thus, these data should be regarded as varying in consistency, reliability, and completeness. The illness information may be too unreliable to permit secular comparisons (i.e., comparison of rates in the same month of different years) due to the revisions in methodology. Illness information was only collected for a three month period (July-September) in 1980. Information which was collected during this period of time was at best incomplete, since many households did not provide any illness data due to collection problems. Information which was obtained between April and September 1981 was more complete but should still be regarded with caution. Also of note is the fact that there is no baseline information available for the October to March interval of time. Therefore, interpretation of illness rates from October 1982 to March 1983 is limited since there is no basis for comparison. Finally, for purposes of consistency, AEI values from the spring of 1982 were used to classify participants into the three exposure levels and two exposure groups during the baseline period. The subpopulations in the ''spring'' exposure levels differ slightly from the subpopulations in the ''summer'' exposure levels (The high exposure level is comprised of essentially the same participants, but the low and intermediate populations shift dramatically between ''spring'' and ''summer.''). Thus, two slightly different populations are being compared when the irrigation year illness rates (based on ''summer'' exposure levels) are compared to baseline rates (based on ''spring'' exposure levels) for the same monthly intervals.

Illness incidence density ratios and their associated test-based 90% and 95% confidence intervals were calculated as described in Section 4J. These ratios and associated confidence intervals were used to identify the consistent patterns in the data and to identify stable ratios (i.e., those for which the confidence intervals were tight). It should be emphasized that the various problems with the illness data limit the extent to which these results can be extrapolated or directly compared to data from other studies.

Table 54 summarizes the monthly incidence densities by type of acute illness and by exposure level. Table 55 summarizes the same information by exposure group. Cases where the 90% or 95% confidence interval for the incidence density ratio did not include the value 1 have been indicated, provided the expected illness incidence in each exposure category was 2.0 or more. Figures 19-26 present the total acute illness and respiratory illness rates from Table 54 in a bar graph format. Tables 56 and 57 summarize the prevalence density rates. Since the prevalence density rates followed a trend similar to the incidence density rates, this information is not presented in a graphic format.

Baseline

Illness information was collected between July and September in 1980. The high exposure level experienced the highest rate of illness during the month of July: the illness rate in the high exposure level was twice the rate of both the low and intermediate exposure levels. Both ratios of incidence densities were found to be stable and possibly significant using 90% confidence intervals. The low exposure level experienced the highest rate of illness during August and September.

Illness information was collected from April-September in 1981. The high exposure level had the highest rate of illness during May and July. Illnesses reported in May and June had symptoms which were primarily gastrointestinal in nature. Although the rate of new GI illnesses appeared to be higher in both the high and intermediate (primarily Wilson) exposure levels, the prevalence density information in Table 56 indicates that the

TABLE 54. MONTHLY INCIDENCE DENSITY OF SELF-REPORTED ILLNESSES BY TYPE OF ILLNESS AND EXPOSURE LEVEL
[Number of New Illnesses Per 1000 Person-days]
[Number of New Illnesses Indicated in Brackets]

	Total acute			Respiratory			GI			Other acute		
	Low Exp Level	Med Exp Level	High Exp Level	Low Exp Level	Med Exp Level	High Exp Level	Low Exp Level	Med Exp Level	High Exp Level	Low Exp Level	Med Exp Level	High Exp Level
1980												
Jul	4.9[13]	5.2[23]	9.9[10][a,b]	2.7[7]	1.6[7]	5.0[5][c]	1.5[4]	2.0[9]	2.0[2]	0.8[2]	1.6[7]	3.0[3]
Aug	7.0[18]	3.2[15]	3.2[3]	3.5[9]	1.9[9]	2.1[2]	2.3[6]	0.6[3]	0.0[0]	1.2[3]	0.6[3]	1.1[1]
Sep	7.8[31]	2.0[13]	5.0[6][a]	3.5[14]	1.4[9]	1.7[2]	3.3[13]	0.6[4]	1.7[2]	1.0[4]	0.0[0]	1.7[2]
1981												
Apr	9.4[29]	5.2[27]	6.3[7]	6.5[20]	4.0[21]	1.8[2]	2.3[7]	0.8[4]	1.8[2]	0.6[2]	0.4[2]	2.7[3]
May	4.4[13]	5.2[27]	7.7[8]	1.4[4]	2.7[14]	1.0[1]	2.0[6]	1.7[9]	4.8[5][a]	1.0[3]	0.8[4]	1.9[2]
Jun	1.7[5]	2.6[13]	1.0[1]	0.0[0]	0.8[4]	0.0[0]	1.4[4]	1.6[8]	1.0[1]	0.3[1]	0.2[1]	0.0[0]
Jul	5.0[15]	0.8[4]	6.1[6]	0.7[2]	0.4[2]	3.1[3]	4.0[12]	0.2[1]	1.0[1]	0.3[1]	0.2[1]	2.0[2]
Aug	5.2[12]	0.7[3]	1.1[1]	2.6[6]	0.7[3]	0.0[0]	1.7[4]	0.0[0]	0.0[0]	0.9[2]	0.0[0]	1.1[1]
Sep	8.0[9]	0.9[2]	6.6[3]	1.8[2]	0.9[2]	4.4[2]	6.3[7]	0.0[0]	0.0[0]	0.0[0]	0.0[0]	2.2[1]
1982												
Jan	8.2[24]	8.4[45]	9.5[10]	7.2[21]	6.5[35]	9.5[10]	1.0[3]	0.7[4]	0.0[0]	0.0[0]	1.1[6]	0.0[0]
Feb	11.4[34]	6.7[35]	13.0[13][c]	7.7[23]	3.4[18]	10.0[10][b]	3.4[10]	2.3[12]	2.0[2]	0.3[1]	1.0[5]	1.0[1]
Mar	8.2[25]	5.9[29]	10.3[11]	6.3[19]	3.2[16]	8.4[9][c]	1.3[4]	1.4[7]	1.9[2]	0.7[2]	1.2[6]	0.0[0]
Apr	11.1[48]	8.7[70]	5.4[9]	7.6[33]	5.7[46]	4.8[8]	2.5[11]	2.5[20]	0.0[0]	0.9[4]	0.5[4]	0.6[1]
May	4.4[13]	4.9[25]	7.3[8]	1.3[4]	2.1[11]	5.5[6][a,d]	2.7[8]	2.3[12]	0.0[0]	0.3[1]	0.4[2]	1.8[2]
Jun	1.9[6]	2.5[12]	4.0[4]	0.3[1]	1.3[6]	2.0[2]	0.3[1]	0.6[3]	2.0[2]	1.3[4]	0.6[3]	0.0[0]
Jul	4.4[13]	9.2[46]	5.6[6]	2.4[7]	3.6[18]	2.8[3]	1.4[4]	3.8[19]	0.9[1]	0.7[2]	1.8[9]	1.9[2]
Aug	4.4[12]	6.9[34]	9.3[8][b]	3.3[9]	3.2[16]	3.5[3]	1.1[3]	3.0[15]	3.5[3]	0.0[0]	0.6[3]	2.3[2]
Sep	11.4[34]	10.8[55]	12.5[12]	6.7[20]	6.5[33]	5.2[5]	3.7[11]	3.0[15]	6.3[6]	1.0[3]	1.4[7]	1.0[1]
Oct	12.9[49]	11.4[75]	4.8[7]	8.2[31]	4.9[32]	4.8[7]	4.0[15]	5.0[33]	0.0[0]	0.8[3]	1.5[10]	0.0[0]
Nov	17.3[25]	10.8[27]	15.6[15]	13.8[20]	6.4[16]	10.4[10]	2.8[4]	3.2[8]	1.0[1]	0.7[1]	1.2[3]	4.2[4]
Dec	3.8[3]	6.3[8]	11.2[6]	0.0[0]	4.0[5]	9.3[5][d]	1.3[1]	2.4[3]	1.9[1]	2.6[2]	0.0[0]	0.0[0]
1983												
Jan	14.6[22]	11.9[26]	9.2[11]	12.6[19]	10.1[22]	7.6[9]	2.0[3]	1.4[3]	0.0[0]	0.0[0]	0.5[1]	1.7[2]
Feb	11.0[17]	15.1[34]	2.5[3]	7.1[11]	9.3[21]	1.6[2]	3.2[5]	4.4[10]	0.8[1]	0.6[1]	1.3[3]	0.0[0]
Mar	6.9[10]	10.0[22]	5.1[6]	6.2[9]	8.6[19]	2.5[3]	0.0[0]	0.0[0]	0.8[1]	0.7[1]	0.9[2]	1.7[2]
Apr	7.7[17]	3.2[10]	7.5[13][c]	5.9[13]	2.3[7]	5.2[9][a]	1.4[3]	0.6[2]	0.6[1]	0.5[1]	0.3[1]	1.7[3]
May	6.6[10]	5.7[12]	5.8[7]	4.7[7]	1.9[4]	5.8[7][a]	1.3[2]	3.3[7]	0.0[0]	0.7[1]	0.5[1]	0.0[0]
Jun	7.1[8]	3.0[6]	6.9[7]	2.7[3]	3.0[6]	4.9[5]	1.8[2]	0.0[0]	0.0[0]	2.7[3]	0.0[0]	2.0[2]
Jul	6.6[7]	4.3[9]	7.1[7]	3.8[4]	1.9[4]	6.1[6][c]	1.9[2]	1.4[3]	1.0[1]	0.9[1]	1.0[2]	0.0[0]
Aug	2.8[3]	3.8[8]	2.9[3]	0.0[0]	2.4[5]	1.9[2]	1.9[2]	1.4[3]	1.0[1]	0.9[1]	0.0[0]	0.0[0]
Sep	7.6[13]	8.5[26]	7.5[11]	4.7[8]	3.6[11]	2.1[3]	1.8[3]	4.6[14]	4.1[6]	1.2[2]	0.3[1]	1.4[2]

a The 90% confidence interval of the incidence density ratio of high-to-intermediate exposure levels does not include the value 1.
b The 90% confidence interval of the incidence density ratio of high-to-low exposure levels does not include the value 1.
c The 95% confidence interval of the incidence density ratio of high-to-intermediate exposure levels does not include the value 1.
d The 95% confidence interval of the incidence density ratio of high-to-low exposure levels does not include the value 1.

TABLE 55. MONTHLY INCIDENCE DENSITY OF SELF-REPORTED ILLNESSES BY TYPE OF ILLNESS AND EXPOSURE GROUP

(Number of New Illnesses Per 1000 Person-days)
[Number of New Illnesses Indicated in Brackets]

	Total Acute		Respiratory		GI		Other acute		Chronic	
	Low exp group	High exp group	Low exp group	High exp group	Low exp group	High exp group	Low exp group	High exp group	Low exp group	High exp group
1980										
Jul	4.9[27]	7.5[19]	1.8[10]	3.5[9]	1.5[8]	2.7[7]	1.6[9]	1.2[3]	0.2[1]	0.4[1]
Aug	4.8[27]	3.6[9]	2.9[16]	1.6[4]	1.3[7]	0.8[2]	0.7[4]	1.2[3]	0.0[0]	0.0[0]
Sep	5.0[42]	2.5[8]	2.5[21]	1.2[4]	2.0[17]	0.6[2]	0.5[4]	0.6[2]	0.1[1]	0.3[1]
1981										
Apr	7.8[50]	4.3[13]	6.0[38]	1.6[5]	1.6[10]	1.0[3]	0.3[2]	1.6[5][a]	0.0[0]	0.0[0]
May	4.8[30]	6.2[18]	2.4[15]	1.4[4]	1.6[10]	3.5[10][b]	0.8[5]	1.4[4]	0.2[1]	0.3[1]
Jun	2.7[17]	0.8[2]	0.6[4]	0.0[0]	1.8[11]	0.8[2]	0.3[2]	0.0[0]	0.0[0]	0.0[0]
Jul	2.9[18]	2.7[7]	0.6[4]	1.1[3]	1.9[12]	0.8[2]	0.3[2]	0.8[2]	0.0[0]	0.0[0]
Aug	3.1[15]	0.4[1]	1.8[9]	0.0[0]	0.8[4]	0.0[0]	0.4[2]	0.4[1]	0.0[0]	0.0[0]
Sep	3.8[10]	3.1[4]	1.2[3]	2.3[3]	2.7[7]	0.0[0]	0.0[0]	0.8[1]	0.0[0]	0.0[0]
1982										
Jan	8.0[51]	9.4[28]	6.6[42]	8.0[24]	0.9[6]	0.3[1]	0.5[3]	1.0[3]	0.0[0]	0.0[0]
Feb	8.3[53]	10.3[29]	5.3[34]	6.0[17]	2.2[14]	3.6[10]	0.8[5]	0.7[2]	0.0[0]	0.0[0]
Mar	7.1[45]	7.3[20]	4.9[31]	4.8[13]	1.3[8]	1.8[5]	0.9[6]	0.7[2]	0.0[0]	0.0[0]
Apr	9.2[88]	8.7[39]	6.0[57]	6.7[30]	2.6[25]	1.3[6]	0.6[6]	0.7[3]	0.0[0]	0.2[1]
May	4.5[28]	6.1[18]	1.9[12]	3.0[9]	2.4[15]	1.7[5]	0.2[1]	1.3[4]	0.0[0]	0.0[0]
Jun	2.2[16]	3.7[6]	0.7[5]	2.5[4]	0.6[4]	1.2[2]	1.0[7]	0.0[0]	0.0[0]	0.0[0]
Jul	7.6[55]	5.7[10]	3.3[24]	2.3[4]	3.2[23]	0.6[1]	1.1[8]	2.8[5][b]	0.0[0]	0.0[0]
Aug	6.3[44]	6.5[10]	3.6[25]	1.9[3]	2.3[16]	3.2[5]	0.4[3]	1.3[2]	0.0[0]	0.0[0]
Sep	11.3[83]	10.7[18]	7.1[52]	3.6[6]	3.1[23]	5.3[9]	1.1[8]	1.8[3]	0.0[0]	0.0[0]
Oct	11.8[112]	8.2[19]	6.4[61]	3.9[9]	4.3[41]	3.0[7]	1.1[10]	1.3[3]	0.0[0]	0.0[0]
Nov	13.1[49]	15.3[18]	9.4[35]	9.3[11]	2.7[10]	2.5[3]	1.1[4]	3.4[4]	0.0[0]	0.0[0]
Dec	4.7[9]	11.9[8][b]	2.1[4]	8.9[6][a]	1.6[3]	3.0[2]	1.1[2]	0.0[0]	0.0[0]	0.0[0]
1983										
Jan	13.4[41]	9.9[16]	11.4[35]	8.3[15]	2.0[6]	0.0[0]	0.0[0]	1.7[3]	0.0[0]	0.0[0]
Feb	13.1[41]	6.9[13]	8.3[26]	4.3[8]	3.8[12]	2.1[4]	1.0[3]	0.5[1]	0.0[0]	1.1[2]
Mar	9.7[29]	5.0[9]	8.7[26]	2.8[5]	0.0[0]	0.6[1]	1.0[3]	1.1[2]	0.0[0]	0.0[0]
Apr	5.2[23]	6.4[17]	3.8[17]	4.5[12]	1.1[5]	0.4[1]	0.2[1]	1.5[4]	0.0[0]	0.0[0]
May	7.0[21]	4.4[8]	3.7[11]	3.8[7]	3.0[9]	0.0[0]	0.3[1]	0.5[1]	0.0[0]	0.0[0]
Jun	4.6[14]	6.1[7]	3.0[9]	4.4[5]	0.7[2]	0.0[0]	1.0[3]	1.7[2]	0.0[0]	0.0[0]
Jul	5.0[15]	7.0[8]	2.7[8]	5.3[6]	1.3[4]	1.8[2]	1.0[3]	0.0[0]	0.0[0]	0.0[0]
Aug	3.3[10]	3.3[4]	1.7[5]	1.7[2]	1.3[4]	1.7[2]	0.3[1]	0.0[0]	0.0[0]	0.0[0]
Sep	8.2[37]	7.6[13]	4.2[19]	1.8[3]	3.3[15]	4.7[8]	0.7[3]	1.2[2]	0.0[0]	0.0[0]

[a] The 95% confidence interval of the incidence density ratio of high-to-low exposure groups does not include the value 1.

[b] The 90% confidence interval of the incidence density ratio of high-to-low exposure groups does not include the value 1.

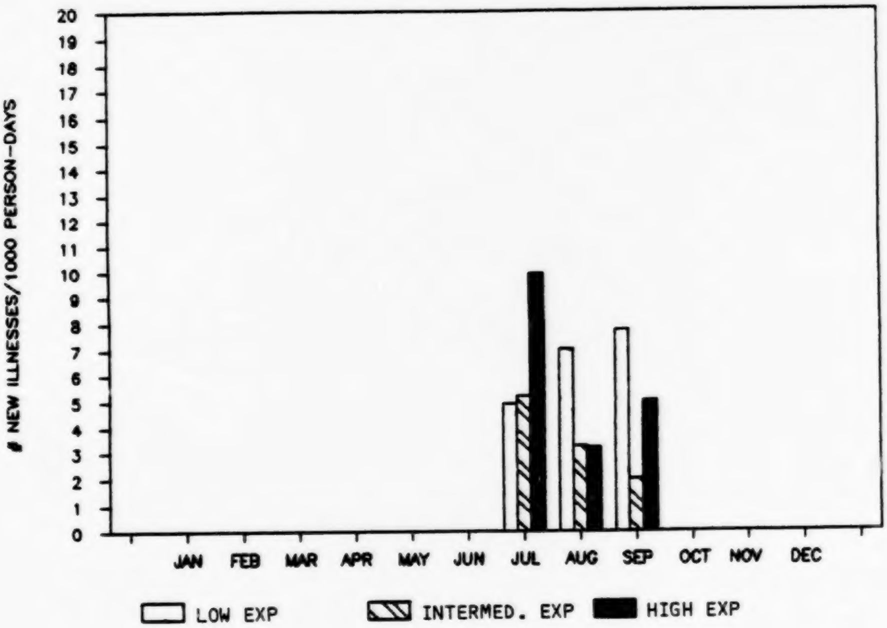


Figure 19. Incidence density rates by exposure level for total acute illness by month--1980

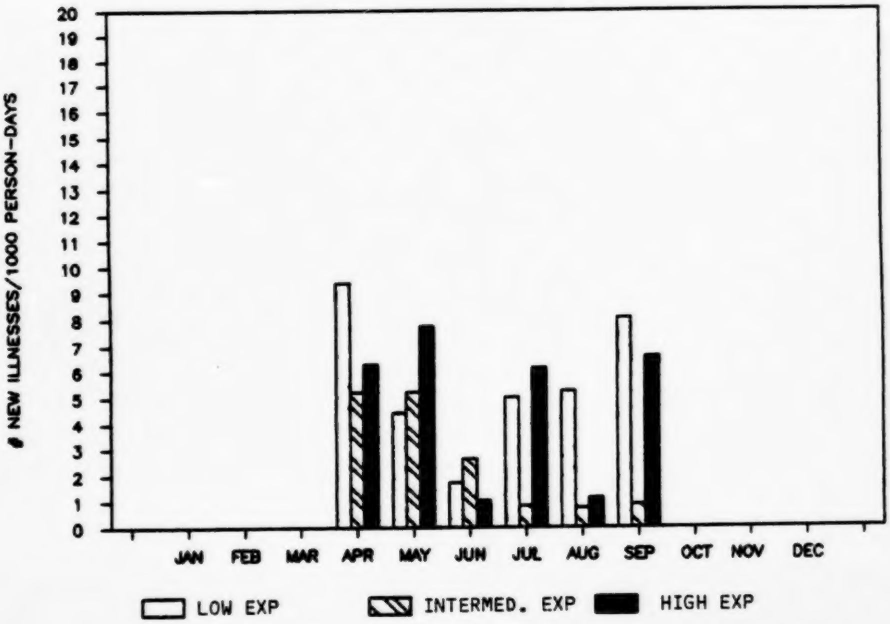


Figure 20. Incidence density rates by exposure level for total acute illness by month--1981

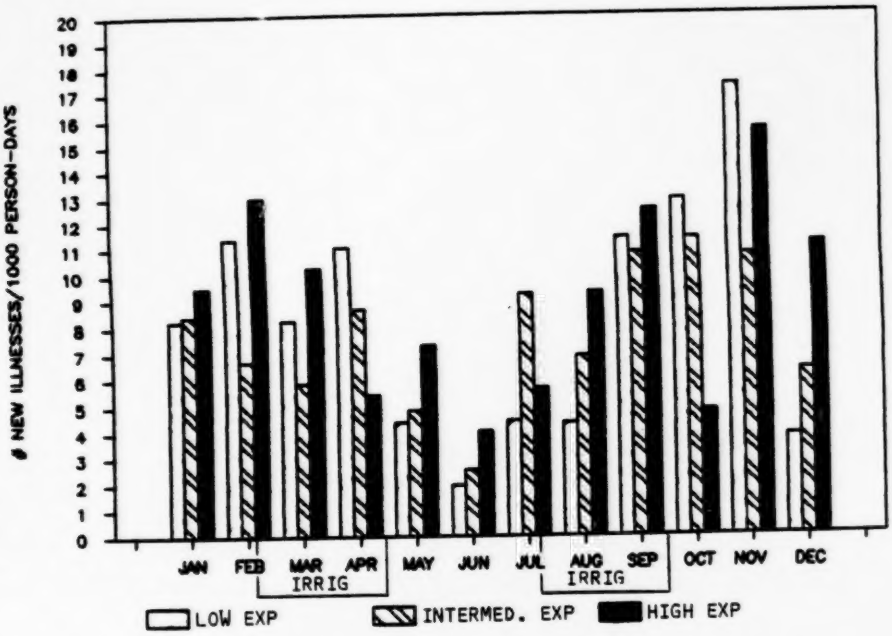


Figure 21. Incidence density rates by exposure level for total acute illness by month--1982

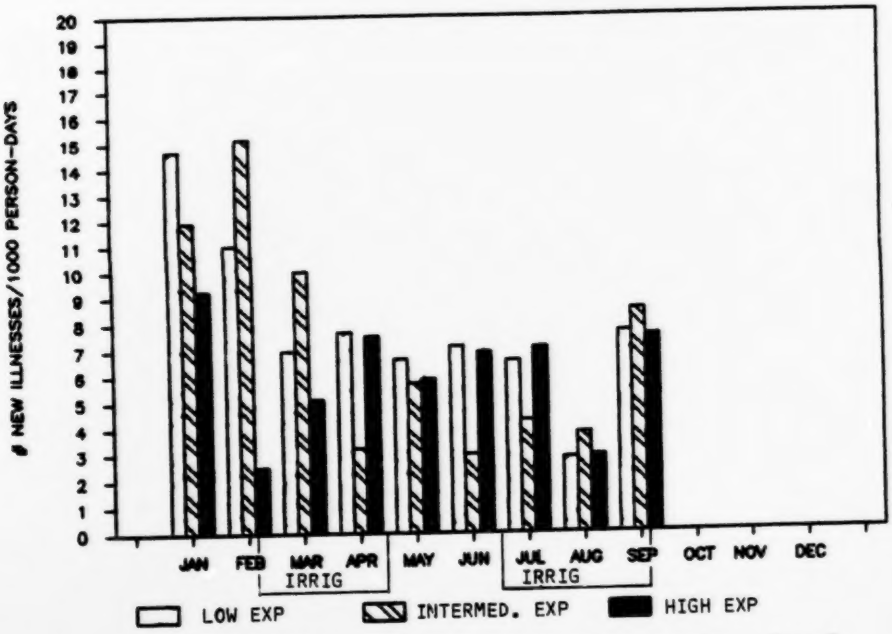


Figure 22. Incidence density rates by exposure level for total acute illness by month--1983

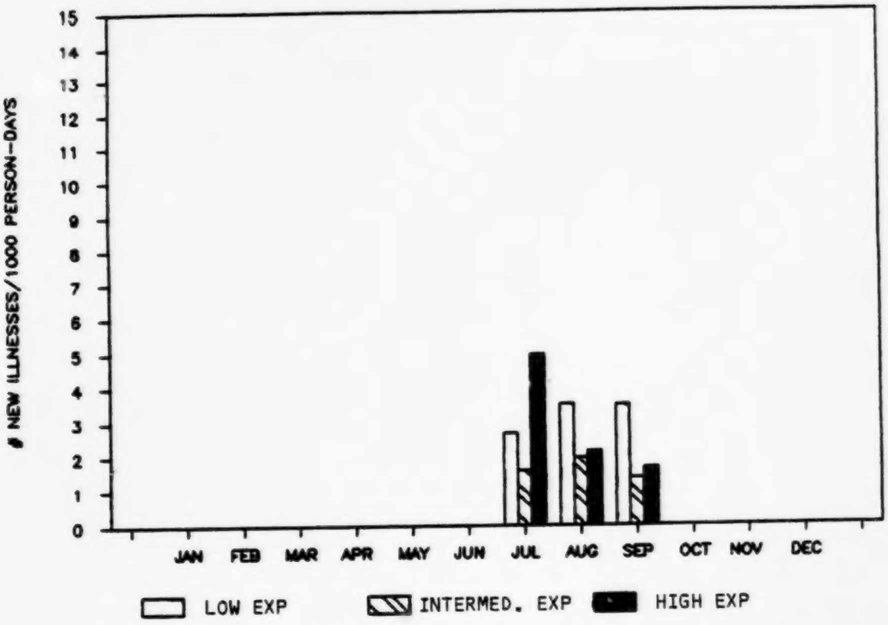


Figure 23. Incidence density rates by exposure level for respiratory illness by month--1980

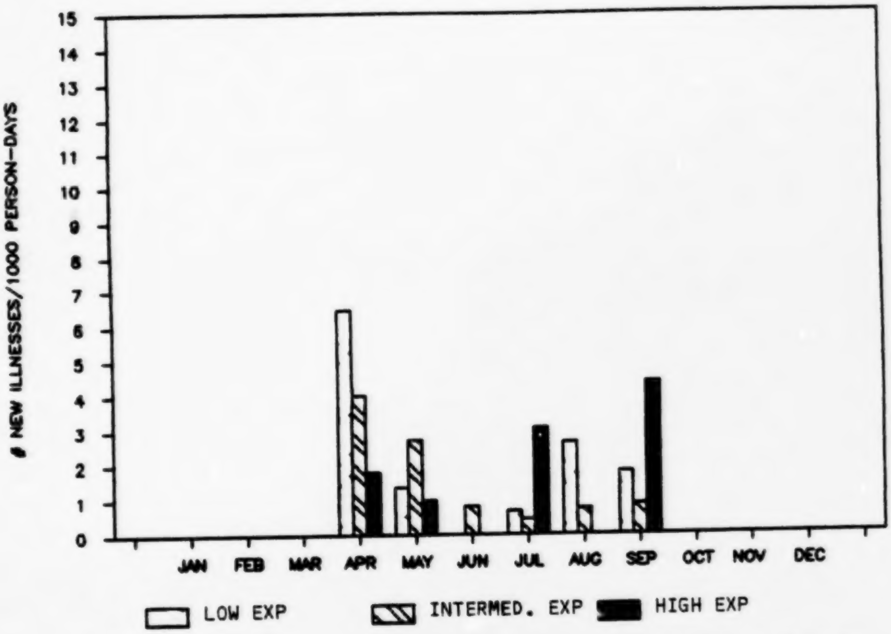


Figure 24. Incidence density rates by exposure level for respiratory illness by month--1981

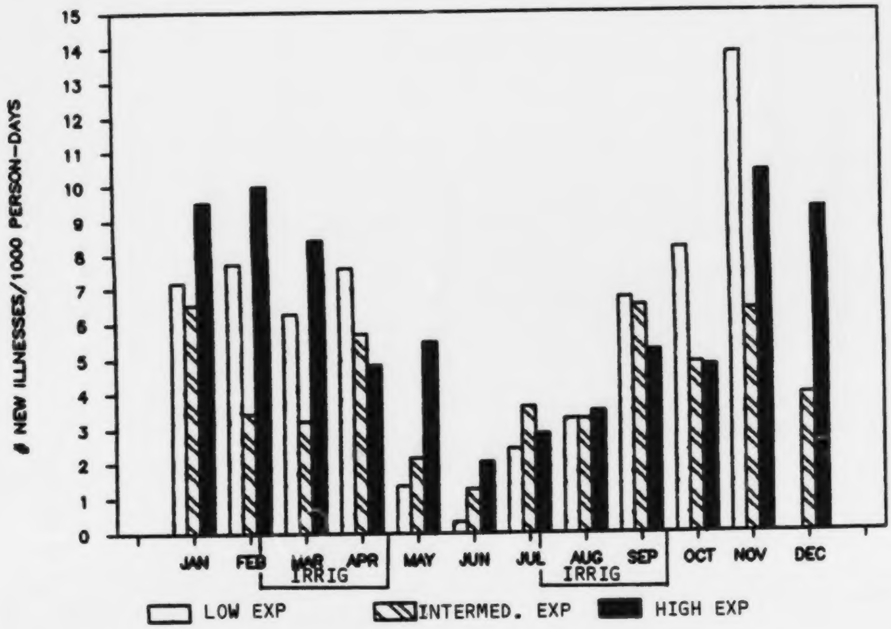


Figure 25. Incidence density rates by exposure level for respiratory illness by month--1982

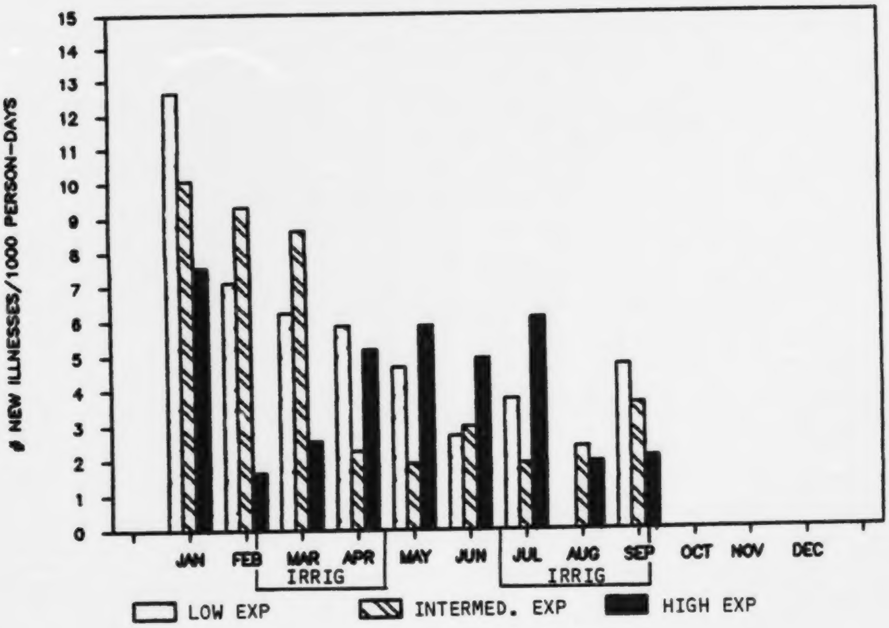


Figure 26. Incidence density rates by exposure level for respiratory illness by month--1983

TABLE 56. MONTHLY PREVALENCE DENSITY OF SELF-REPORTED ILLNESSES BY TYPE OF ILLNESS AND EXPOSURE LEVEL
(Number of New Illnesses Per 1000 Person-days)
[Number of New Illnesses Indicated in Brackets]

	Total acute			Respiratory			GI			Other acute		
	Low Exp Level	Med Exp Level	High Exp Level	Low Exp Level	Med Exp Level	High Exp Level	Low Exp Level	Med Exp Level	High Exp Level	Low Exp Level	Med Exp Level	High Exp Level
1980												
Jul	58.5[157]	24.0[106]	52.6[53]	8.7[23]	8.2[36]	35.7[36]	6.1[16]	8.4[37]	5.0[5]	44.7[118]	7.5[33]	11.9[12]
Aug	24.2[62]	16.5[76]	27.7[26]	14.1[36]	11.9[55]	6.4[6]	7.0[18]	2.4[11]	0.0[0]	3.1[8]	2.2[10]	21.3[20]
Sep	52.9[211]	14.1[92]	23.5[28]	32.1[128]	8.6[56]	6.7[8]	16.3[65]	2.9[19]	3.4[4]	4.5[18]	2.6[17]	13.4[16]
1981												
Apr	51.4[159]	32.8[171]	20.5[23]	38.5[119]	26.3[137]	6.3[7]	10.0[31]	4.0[21]	1.8[2]	2.9[9]	2.5[13]	12.5[14]
May	62.0[183]	26.1[135]	36.7[38]	8.8[26]	17.2[89]	7.7[8]	42.0[124]	4.4[23]	11.6[12]	7.5[22]	4.4[23]	17.4[18]
Jun	11.0[32]	11.6[58]	4.1[4]	0.0[0]	2.0[10]	0.0[0]	9.6[28]	8.0[40]	4.1[4]	1.4[4]	1.6[8]	0.0[0]
Jul	67.9[204]	4.9[24]	45.8[45]	5.0[15]	2.3[11]	20.4[20]	60.6[182]	0.6[3]	3.1[3]	2.3[7]	2.1[10]	22.4[22]
Aug	21.8[50]	2.9[12]	7.8[7]	8.3[19]	2.9[12]	0.0[0]	5.2[12]	0.0[0]	0.0[0]	8.3[19]	0.0[0]	7.8[7]
Sep	35.7[40]	7.3[17]	54.7[25]	8.9[10]	7.3[17]	24.1[11]	26.8[30]	0.0[0]	0.0[0]	0.0[0]	0.0[0]	30.6[14]
1982												
Jan	50.7[148]	46.2[248]	77.1[81]	48.3[141]	39.5[212]	77.1[81]	2.4[7]	2.2[12]	0.0[0]	0.0[0]	4.5[24]	0.0[0]
Feb	65.1[194]	46.6[244]	85.9[86]	50.3[150]	35.7[187]	85.9[86]	8.7[26]	6.7[35]	6.0[6]	6.0[18]	4.2[22]	4.0[4]
Mar	64.9[197]	33.3[165]	75.6[81]	60.3[183]	18.4[91]	71.9[77]	1.6[5]	5.7[28]	3.7[4]	3.0[9]	9.3[46]	0.0[0]
Apr	63.6[276]	59.6[480]	33.8[56]	53.0[230]	45.0[363]	30.8[51]	6.0[26]	7.4[60]	0.0[0]	4.6[20]	7.1[57]	3.0[5]
May	40.1[119]	29.7[153]	47.6[52]	23.6[70]	14.6[75]	32.9[36]	10.8[32]	10.5[54]	0.0[0]	5.7[17]	4.7[24]	14.6[16]
Jun	13.3[41]	24.8[118]	71.6[71]	7.4[23]	12.6[60]	42.3[42]	0.3[1]	1.5[7]	5.0[5]	5.5[17]	10.7[51]	24.2[24]
Jul	36.1[106]	75.9[379]	47.0[50]	9.9[29]	22.4[112]	16.9[18]	3.1[9]	18.4[92]	7.5[8]	23.2[68]	35.0[175]	22.6[24]
Aug	23.7[65]	61.9[305]	54.5[47]	9.5[26]	27.2[134]	17.4[15]	4.0[11]	13.0[64]	15.1[13]	10.2[28]	21.7[107]	22.0[19]
Sep	76.0[226]	80.7[410]	50.1[48]	48.7[145]	48.4[246]	30.3[29]	9.7[29]	10.6[54]	17.7[17]	17.5[52]	21.7[110]	2.1[2]
Oct	78.6[298]	80.0[527]	51.3[75]	59.6[226]	37.2[245]	51.3[75]	11.6[44]	20.5[135]	0.0[0]	7.4[28]	22.3[147]	0.0[0]
Nov	144.5[209]	77.0[193]	99.8[96]	128.6[186]	51.8[130]	83.2[80]	10.4[15]	13.6[34]	2.1[2]	5.5[8]	11.6[29]	14.6[14]
Dec	85.9[67]	81.7[103]	215.0[115]	66.7[52]	65.9[83]	129.0[69]	9.0[7]	15.9[20]	5.6[3]	10.3[8]	0.0[0]	80.4[43]
1983												
Jan	127.7[192]	79.2[173]	52.9[63]	115.0[173]	71.5[156]	47.1[56]	5.3[8]	5.0[11]	0.0[0]	7.3[11]	2.7[6]	5.9[7]
Feb	169.7[262]	115.6[260]	36.3[44]	129.5[200]	84.4[190]	12.4[15]	22.7[35]	26.7[60]	0.8[1]	17.5[27]	4.4[10]	23.1[28]
Mar	113.8[164]	90.6[199]	49.9[59]	104.1[150]	86.9[191]	33.0[39]	4.9[7]	0.0[0]	0.8[1]	4.9[7]	2.3[5]	16.1[19]
Apr	52.3[116]	31.5[98]	65.9[114]	45.1[100]	23.8[74]	40.4[70]	5.0[11]	1.3[4]	1.2[2]	2.3[5]	6.4[20]	24.3[42]
May	67.2[101]	37.0[78]	44.3[53]	59.8[90]	19.5[41]	44.3[53]	6.6[10]	12.8[27]	0.0[0]	0.7[1]	4.7[10]	0.0[0]
Jun	52.5[59]	14.3[29]	70.0[71]	24.0[27]	14.3[29]	54.2[55]	2.7[3]	0.0[0]	0.0[0]	25.8[29]	0.0[0]	15.8[16]
Jul	48.8[52]	21.9[46]	34.4[34]	36.6[39]	13.3[28]	33.4[33]	1.9[2]	3.8[8]	1.0[1]	10.3[11]	4.8[10]	0.0[0]
Aug	13.2[14]	15.5[33]	30.8[32]	1.9[2]	10.4[22]	27.9[29]	7.5[8]	5.2[11]	2.9[3]	3.8[4]	0.0[0]	0.0[0]
Sep	55.8[95]	31.7[97]	30.1[44]	38.2[65]	15.7[48]	6.2[9]	8.2[14]	14.1[43]	8.2[12]	9.4[16]	2.0[6]	15.7[23]

TABLE 57. MONTHLY PREVALENCE DENSITY OF SELF-REPORTED ILLNESSES BY TYPE OF ILLNESS AND EXPOSURE GROUP
[Number of New Illnesses Per 1000 Person-days]
[Number of New Illnesses Indicated in Brackets]

	Total Acute		Respiratory		GI		Other acute		Chronic	
	Low exp group	High exp group	Low exp group	High exp group	Low exp group	High exp group	Low exp group	High exp group	Low exp group	High exp group
1980										
Jul	40.3[222]	36.9[94]	19.2[35]	23.5[60]	6.5[36]	8.6[22]	27.4[151]	4.7[12]	0.7[4]	0.0[0]
Aug	19.6[110]	21.4[54]	36.4[77]	7.9[20]	3.8[21]	3.2[8]	2.1[12]	10.3[26]	0.0[0]	0.0[0]
Sep	33.6[283]	14.7[48]	51.0[164]	8.6[28]	10.0[84]	1.2[4]	4.2[35]	4.9[16]	3.9[33]	0.0[0]
1981										
Apr	45.0[287]	21.6[66]	44.2[227]	11.8[36]	8.0[51]	1.0[3]	1.4[9]	8.8[27]	0.0[0]	0.0[0]
May	42.4[266]	31.1[90]	34.9[86]	12.8[37]	20.9[131]	9.7[28]	6.1[38]	8.6[25]	0.0[0]	4.8[14]
Jun	12.7[79]	5.7[15]	12.4[10]	0.0[0]	9.9[62]	3.8[10]	1.1[7]	1.9[5]	0.0[0]	0.0[0]
Jul	36.2[225]	18.3[48]	41.5[26]	7.6[20]	29.3[182]	2.3[6]	2.7[17]	8.4[22]	0.0[0]	0.0[0]
Aug	12.7[62]	2.9[7]	82.7[31]	0.0[0]	2.5[12]	0.0[0]	3.9[19]	2.8[7]	0.0[0]	0.0[0]
Sep	17.7[46]	27.8[36]	60.4[16]	17.0[22]	11.5[30]	0.0[0]	0.0[0]	10.8[14]	0.0[0]	0.0[0]
1982										
Jan	48.0[305]	57.6[172]	21.9[280]	51.6[154]	2.0[13]	2.0[6]	1.9[12]	4.0[12]	0.0[0]	0.0[0]
Feb	56.8[364]	60.4[170]	19.9[284]	49.4[139]	6.9[44]	8.2[23]	5.6[36]	2.8[8]	0.0[0]	0.0[0]
Mar	48.4[306]	50.1[137]	6.4[245]	38.8[106]	2.5[16]	7.7[21]	7.1[45]	3.7[10]	0.0[0]	0.0[0]
Apr	56.9[544]	59.7[268]	13.8[415]	51.0[229]	7.3[70]	3.6[16]	6.2[59]	5.1[23]	0.0[0]	2.7[12]
May	31.8[198]	42.4[126]	19.5[113]	22.9[68]	10.6[66]	6.7[20]	3.0[19]	12.8[38]	0.0[0]	0.0[0]
Jun	17.8[129]	63.0[101]	35.6[56]	43.0[69]	1.1[8]	3.1[5]	9.0[65]	16.8[27]	0.0[0]	0.0[0]
Jul	58.3[422]	64.3[113]	13.7[124]	19.9[35]	14.0[101]	4.6[8]	27.2[197]	39.8[70]	0.0[0]	0.0[0]
Aug	46.9[328]	57.5[89]	1.6[142]	21.3[33]	9.3[65]	14.8[23]	17.3[121]	21.3[33]	0.0[0]	0.0[0]
Sep	80.1[587]	57.5[97]	4.2[379]	24.3[41]	10.2[75]	14.8[25]	18.2[133]	18.4[31]	0.0[0]	0.0[0]
Oct	81.8[778]	52.4[122]	6.4[461]	36.5[85]	17.2[164]	6.4[15]	16.1[153]	9.4[22]	0.0[0]	0.0[0]
Nov	104.1[389]	92.5[109]	6.1[309]	73.9[87]	11.5[43]	6.8[8]	9.9[37]	11.9[14]	0.0[0]	0.0[0]
Dec	73.7[140]	214.8[145]	44.1[114]	133.3[90]	9.5[18]	17.8[12]	4.2[8]	63.7[43]	0.0[0]	0.0[0]
1983										
Jan	105.3[323]	58.0[105]	44.3[293]	50.8[92]	6.2[19]	0.0[0]	3.6[11]	7.2[13]	0.0[0]	0.0[0]
Feb	146.7[459]	57.0[107]	38.7[344]	32.5[61]	25.6[80]	8.5[16]	11.2[35]	16.0[30]	0.0[0]	13.3[25]
Mar	112.9[339]	45.7[83]	43.4[320]	33.0[60]	2.3[7]	0.6[1]	4.0[12]	10.5[19]	0.0[0]	0.0[0]
Apr	39.6[175]	57.9[153]	18.1[155]	33.7[89]	3.4[15]	0.8[2]	1.1[5]	23.5[62]	0.0[0]	0.0[0]
May	56.7[169]	34.5[63]	7.7[131]	29.0[53]	12.4[37]	0.0[0]	0.3[1]	5.5[10]	0.0[0]	0.0[0]
Jun	29.1[88]	61.8[71]	17.1[56]	47.9[55]	1.0[3]	0.0[0]	9.6[29]	13.9[16]	0.0[0]	0.0[0]
Jul	31.8[96]	31.6[36]	20.3[67]	29.0[33]	2.7[8]	2.6[3]	7.0[21]	0.0[0]	0.0[0]	0.0[0]
Aug	13.2[40]	32.5[39]	51.7[24]	24.2[29]	4.0[12]	8.3[10]	1.3[4]	0.0[0]	0.0[0]	0.0[0]
Sep	41.2[186]	29.3[50]	48.5[113]	5.3[9]	11.3[51]	10.5[18]	4.9[22]	13.5[23]	0.0[0]	0.0[0]

low exposure level was reporting GI illnesses which continued over longer durations during May. Yersinia enterocolitica was isolated from the routine stool specimens of two Wilson children in June and from the routine stool specimen of another Wilson child in July (see Table 70). None of these participants resided in the same household or were related to each other. Illnesses reported during July-September were primarily respiratory and occurred mostly in rural areas.

In summarizing the self-reported illnesses which were reported during the primarily ''summer'' months of the baseline period, the high exposure level was found to have the highest rate of self-reported illness during 3 of the 9 months investigated. The low exposure level had the highest rate of illness during 5 of the 9 months. The intermediate level, primarily the city of Wilson, had the highest rate of illness during only 1 of the 9 baseline months investigated (i.e., June 1981); it may have been associated with the GI illness which affected the entire study area. It appears, therefore, that the participants living in rural areas tended to report a higher rate of illness during the baseline period. Furthermore, the rural residents in the low exposure area tended to report a higher rate of illness than did the rural residents in the high exposure area. Given the fact that the majority of the ''susceptibles'' (the lower socioeconomic status families and the families with children) resided in the city of Wilson, this result would not have been predicted.

Irrigation-1982

With the exception of the last 2 weeks of the year, illness information was collected during all of 1982. Only the sentinel families were contacted for information after October 23. The high exposure level participants reported the highest rate of total acute illness in 8 of the 12 months. The low exposure level reported the highest rate of total acute illness in 3 of the 12 months in 1982. The intermediate exposure level reported the highest rate of total acute illness during 1 month in 1982.

A high rate of respiratory illness was reported for all three exposure levels prior to the initial irrigation, with the highest rates of illness being reported in the low exposure level. During the 2-week interval after the onset of irrigation (February 14-27, 1982), the rates of illness (primarily respiratory) reported by the high exposure level participants increased to a level twice as high as the illness rate reported by the low exposure level participants and three times the rate reported by the intermediate exposure level participants. The incidence density ratio between the high and intermediate exposure levels was found to be significant when the 95% confidence interval was calculated. The high-to-low exposure level ratio was not found to be stable. This illness pattern continued through March.

In April, the rate of illness in the high exposure level decreased as the illness rate in both the low and intermediate exposure levels increased. While it is possible that the respiratory illnesses which were experienced by the high exposure level after the onset of irrigation were transmitted to the other exposure levels, comparison of April 1982 incidence density rates to April 1981 incidence density rates suggests that April 1982 incidence density rates were not unusual. The prevalence densities for the same periods of time do suggest that the respiratory illnesses reported in April 1982 lasted for a longer period of time.

The incidence density of self-reported illnesses increased in the high exposure level during May, after major irrigation had ceased. This illness pattern is similar to the pattern observed in May 1981, except that the high exposure level participants reported respiratory symptoms while the low and intermediate exposure participants reported respiratory and GI symptoms. A Norwalk viral particle was identified in an illness fecal specimen collected from an 18-month-old participant from Wilson during this period of time (see Table 66). The rates of illness decreased in June; however, the high exposure level continued to report the highest illness rate. Of note is the fact that the prevalence of self-reported acute illness in June 1982 was quite high when compared to June 1981, especially in the high and intermediate exposure groups. Only 58 person-days of total acute illness were reported for the intermediate exposure level in June 1981; 118 person-days of illness were reported for the same period in June 1982. Four person-days of illness were reported for the high exposure level in June 1981; 71 person-days of illness were reported in June 1982. The prevalence of illness in June 1982 may have been associated with the heavy rainfall which occurred from the last week of May through June 1982 (see Table 47) and the resultant flooding which appeared to have contaminated many rural drinking water wells (Table 46). The intermediate exposure level experienced a sharp increase in incidence and prevalence density rates during the month of July. The illness observed in the intermediate exposure level (primarily the northern section of Wilson) during July appears to be unusual. However, since irrigation did not commence until July 21, the unexpected increases cannot be attributed to wastewater aerosol exposure. It should be noted that enteric Gram-negative bacteria (EGNB) were first isolated at unusually high levels from the throats of a family living in the northern sector of Wilson during July and prior to the summer irrigation. This unexpected EGNB phenomenon, which was also observed by September in both ill and healthy participants throughout the study area and lasted into October, is discussed in Section 5.F.

The illness rate increased in the high exposure level during August after the start of summer irrigation. Using the 90% confidence interval, the incidence density ratio of the high-to-low exposure level was found to be stable and possibly significant. Three weeks after irrigation commenced (during August 15-28, 1982), the incidence density rate of total acute illness in the high exposure level was twice the rate found in the low and intermediate exposure levels. Using the 90% confidence interval, the incidence density ratio of the high-to-low exposure levels was found to be possibly significant for total acute illnesses in this 2-week period. When prevalence density rates for total acute illness in August 1982 are compared to rates for the same month in 1980 and 1981, it can be seen that the low exposure level reported approximately the same rate of person-days of illness during August for all 3 years. The prevalence rate for the intermediate exposure group in 1982 was three times higher than the rate reported in August 1980, and twenty times higher than the rate reported during August 1981. The high exposure level reported a rate twice as high as the rate in 1980, and seven times greater than the rate reported in 1981.

Total acute illness incidence density rates increased for all exposure levels during the month of September. The high exposure level continued to report the highest rate of illness, especially GI illness, during this period of time. The rate of illness in the high exposure level decreased in October after irrigation was completed and then increased in November. Illness rates during this period of time appeared to be quite high, especially in the rural areas. However, an increase in respiratory illnesses was expected during this time of year. The illness rates for all three exposure levels decreased during December. The low exposure level reported the largest decrease in illness; the high exposure level experienced a smaller decrease and reported the highest rate of respiratory illness during this period of time. Symptoms reported by the high exposure level, in combination with the high prevalence density rates, suggest the onset of the ''flu season.'' The respiratory illness incidence density ratio of the high-to-low exposure levels in December was found to be significant.

In summary, it appears that the high exposure level reported the highest monthly rate of total acute illness more frequently during 1982 than in the months observed during 1980 or 1981. The high exposure level reported the highest rate of illness during four distinct periods of time in 1982: after the onset of irrigation in both the spring and the summer, in late spring, and in December. There is no basis for comparing illness rates after the onset of the spring irrigation. Comparison of the rates of illness after the onset of summer irrigation suggests that rates for the high and intermediate exposure groups in August 1982 were much higher than the rates observed during the same period of time in 1980 and 1981. The high rate of illness in May and June occurred after spring irrigation had concluded and followed extremely heavy rainfall. The May 1982 pattern was similar, though not identical, to the May 1981 pattern, but the June prevalence patterns in the high level were very different. Therefore, there is no real evidence that the illnesses which were observed in the late spring were associated with exposure to wastewater aerosols. Finally, the illness episode during December 1982 appeared to be associated with the onset of the ''flu season.''

Irrigation-1983

Illness information was collected from sentinel families between January and September in 1983. The high exposure level reported the highest rate of illness during one of the nine months that were observed (i.e., July 1983). The low and intermediate exposure levels each reported the highest rate of illness for 4 of the 9 months.

A high rate of respiratory illness was observed in January through March. As in December 1982, the prevalence density rates and the reported symptoms suggested that influenza was circulating through the community. The low exposure level participants reported the highest rate of illness in January; the intermediate exposure participants (mainly Wilson residents) reported the highest rate of respiratory illness in February and March.

There was a slight increase in the total acute illness incidence density rate for the high exposure level in March after the onset of irrigation. However, the rate was lower than the rates for low and intermediate exposure levels and lower than the incidence density rates observed in March 1982. The high exposure level illness rate increased again in April, and remained at a consistent level until August. The low and high exposure levels reported approximately the same rate of illness between April and September, with both exposure levels reporting a drop in illness rates in August. The prevalence density rate for the high exposure level did not decrease in parallel with the incidence density rate in August. The intermediate exposure level participants reported a lower rate of illness than the high and low exposure level participants between April and July.

In summary, it does not appear that there was an increase in the illness rates of the high exposure level at the onset of irrigation in either February or July 1983. After the apparent outbreak of influenza had subsided, there appeared to be a higher rate of illness in the rural areas than in Wilson in April, June, and July. The illness rates were similar for all three exposure levels during May, August, and September. The pattern of illness which was observed in 1983 bore little resemblance to the overall illness patterns which were observed in either 1982 or the baseline years.

Discussion

Disease surveillance did not disclose any obvious connection between illness and degree of wastewater exposure. The self-reported illness data varied in consistency, reliability, and completeness over the July 1980-September 1983 period of surveillance, with the better quality data obtained during the years of wastewater irrigation. In addition, self-reports of illness are always subject to respondent bias.

Nevertheless, it is of interest and may be significant that the participants in the high exposure level reported the highest density of illness shortly after the onset of wastewater irrigation, both in spring 1982 and in summer 1982. The excess total acute illness among high exposure level participants during the spring 1982 occurred primarily during February 14-27, 1982, in the initial 2 weeks of wastewater irrigation at the Hancock farm. The extent to which this reflects actual illness vs. reporting bias by high exposure participants has not been ascertained. The high exposure level participants also reported a significant excess of total acute illness in August 1982, primarily during August 15-28 (after more than 3 weeks of wastewater irrigation had elapsed). The high exposure level participants did not report a comparable excess of acute illnesses during either irrigation period in 1983. This pattern of excess illness during both irrigation periods is consistent with the hypothesis of an association of illness with exposure to wastewater irrigation in that the pattern appeared both upon initial wastewater exposure and in the summer 1982 irrigation period which produced highest exposure to microorganisms in the wastewater aerosol (see Table 42). However, the patterns did not persist throughout either irrigation period in 1982. The total acute illness incidence density ratios of the high exposure level to the intermediate and low exposure levels were less than 1.5, both for the entire spring 1982 and summer 1982 irrigation periods. Thus, if not a reporting artifact, the excess rate of illnesses which might be associated with the initial and heaviest periods of microorganism emission from wastewater irrigation was small.

Since the agents which the LISS monitored clinically and serologically show a very high proportion of asymptomatic infection, it is difficult to correlate the self-reported illness data with the infection episodes which were observed. However, it is of interest and probably of health significance that the incidence density of self-reported total acute illness increased among high exposure level participants during the initial and heaviest periods of microorganisms exposure via wastewater irrigation.

F. SURVEILLANCE VIA ILLNESS AND REQUESTED SPECIMENS

To determine the causative agent in self-reported respiratory and gastrointestinal illnesses, the ill participant was asked to submit a throat swab or stool specimen for clinical bacteriologic, virologic and electron microscopic analyses, as appropriate. Acute illness specimens were collected while the participant displayed symptoms. If the specimen was obtained within 1 week after recovery from the symptoms of the illness, it was termed a convalescent illness specimen. Follow-up specimens were also sought to clarify the etiology of unusual bacterial findings; these were termed requested specimens. Unusual illness within a household was investigated using requested specimens as a primary source of information. Three substantive illness investigations were performed in 1982.

Illness Investigations

Salmonella Investigation: Household 540, June-August 1982--

Investigation report--Heavy growth of Salmonella sp. Group C_1 was detected in the routine fecal specimen collected from the father (54001) on June 8, 1982. His prior routine fecal specimen collected on March 31 had contained normal fecal flora. The household was contacted on June 18 to request additional fecal specimens from all five family members and to obtain information concerning the source of the Salmonella infection.

The father reported that he was currently being treated for a bladder infection. He reported no other symptoms which would indicate that he was experiencing a Salmonella infection. Exposure information was similarly negative. He reported no exposure to wastewater and could not recall any unusual activities in the weeks prior to collection of the fecal specimens. He did indicate, however, that heavy rainfall and subsequent runoff had infiltrated the well which was the source of the family's drinking water.

After consultation with the Texas Department of Health, it was also decided that treatment of the father for a Salmonella infection was unnecessary since he was not experiencing any symptoms. LCCIWR was asked to obtain a sample of water from the family's well. No bacterial contamination was found in the well water samples collected. Results of the requested fecal specimens collected from the family on June 22 and 23 indicated normal fecal flora in all family members except the father, whose specimen contained a medium growth of Salmonella sp.

The father reported a flare-up of the bladder infection on June 28. A urine specimen was collected and sent to UTSA on July 1. Insignificant levels of *E. coli* and *Citrobacter* sp. were recovered from this sample only by enrichment.

Follow-up fecal specimens were obtained from all family members on July 13 and forwarded to UTSA. *Salmonella* sp. was isolated at the very light level from the specimen provided by a son, age 17 (54011). No unusual bacteria were found in the specimens provided by the other family members.

Follow-up stool specimens were again collected from the entire family on August 2. All specimens were found to contain normal fecal flora.

A final set of four follow-up fecal specimens was collected on September 15 and 16 from all family members except the father. A possibly significant API Group I infection of the son was indicated by isolation at the heavy level. The specimens provided by the three other family members contained normal fecal flora.

Convalescent-phase blood was obtained from the father on August 11. This serum was paired with acute phase serum which was obtained during the regular blood collection clinic on June 8. UTSA obtained serological confirmation that his infection was to *Salmonella* Group C_1.

Discussion--The *Salmonella* infections experienced by the father in June 1982 and by his son in July 1982 were the only infections by overt enteric bacterial pathogens detected in the study population after wastewater irrigation commenced. The father was being treated for a concurrent bladder infection. However, the *Salmonella* infections experienced by the father and son appear to have been asymptomatic.

Household 540 was located more than 2 km from the Hancock farm. The aerosol exposure index values of both infected participants were low for the summer 1982 irrigation period: AEI=0.48 for the father and AEI=1.61 for his son. The *Salmonella* Group C_1 infection of the father preceded the start of the summer 1982 irrigation and he reported having no exposure to wastewater. The onset of the *Salmonella* infection in the son was presumably between June 22 and July 13, prior to commencement of wastewater irrigation operations on July 21. Since heavy rainfall runoff had recently infiltrated the family's drinking water well, contaminated drinking water remains a possible source of the infections, despite lack of evidence of bacterial contamination of the water. Alternatively, the consumption of contaminated food could be a plausible explanation for the *Salmonella* infection (Benenson, 1975). The genus *Salmonella* has an exceptionally wide host range which would suggest a variety of possible sources. Wastewater aerosol exposure is considered an extremely unlikely source of these *Salmonella* infections.

Enteric Gram-negative Bacteria (EGNB) Investigation: Household 210, June-November 1982--

Investigation report--The mother (21002) reported on June 26, 1982 that her 3-year old son (21012) had a cold which began on June 23. A throat swab was obtained on June 29. The son was placed on antibiotic therapy by his physician on June 30. Laboratory analysis of the throat swab yielded normal flora on blood agar, including E. cloacae at the very light level, but a very light level of Group A streptococci was detected by fluorescent antibody (see Table 58).

The mother was contacted on July 8 and given the results of the son's throat swab. She reported that he had recovered from his cold on July 2. She also reported that she had a cold which commenced on July 7. She recovered from the cold on July 12.

On July 13, the 3-year old and his 7-year old brother (21011) went swimming in the Tahoka public swimming pool. The younger son developed a fever and a sore throat later the same evening. The older son developed a fever on July 17 and complained of a headache and a stomachache. Throat swabs were collected from both boys on July 19. It was reported that both boys recovered from their illnesses on July 24, 1982. Moderate to heavy levels of E. coli and Enterobacter cloacae were found in the throat swabs from both boys, and Klebsiella oxytoca was isolated from the younger son's throat swab (see Table 58).

Due to the unusual nature of the July 19 throat swab results, the entire family was asked to submit additional throat swabs on July 29. High (i.e., heavy or moderate) levels of E. coli and E. cloacae were found in the throat cultures of all family members.

It was reported that the father (21001) slept in the living room in front of the evaporative cooler every night during ''hot spells,'' and that the children frequently played in front of the evaporative cooler during the day. The evaporative cooler water and the family's drinking water were supplied by the Wilson water system. Samples of the family's drinking water and reservoir water from the evaporative cooler were collected and sent to UTSA for bacterial screening on August 9. No fecal bacteria were isolated from either sample. Investigation of other possible bacterial sources were essentially negative. However, it was observed that the family frequently shared drinking glasses and eating utensils. Otherwise, no unusual sanitation problems could be identified.

Requested throat swabs were again collected from the family on August 11 and 13. E. coli, E. cloacae and K. oxytoca were found at moderate levels in the throats of all family members except the older son, who had been at his grandmother's house for the week prior to collection of the throat swabs. Based on this finding, it was recommended that the family make an effort to avoid the practice of sharing eating utensils in order to reduce spreading of these fecal bacteria among family members.

The father reported a sore throat and cold which began on August 21 and ended on September 1. He reported that he was taking antibiotics for the condition; however, he had not consulted a physician. A throat swab was obtained on August 30 and forwarded to UTSA for analysis. A moderate growth of E. cloacae was recovered from this swab, but not the Group A streptococcus.

TABLE 58. BACTERIOLOGY THROAT SWAB SERIES FOR DONORS WITH MODERATE OR HEAVY LEVELS OF ENTERIC GRAM-NEGATIVE BACTERIA IN AN ILLNESS THROAT SWAB

Donor ID	Age on 6-30-82	Throat swab category[a]	Specimen collection date	Clinical bacteriology results		
				Abnormal flora?	Gram-negative bacteria (level of growth[b])	Other abnormal flora
Household 210						
21001	32	R	7-29-82	Yes	E. coli (H) E. cloacae (M)	
		R	8-11-82	Yes	E. cloacae (M) E. coli (M) K. oxytoca (M)	
		A	8-30-82	Yes	E. cloacae (M)	
		R	9-15-82	Yes	E. cloacae (H)	
21002	28	R	7-29-82	Yes	E. cloacae (M) E. coli (M)	
		R	8-11-82	Yes	K. oxytoca (M)	
		A	9-14-82	No		
		R	11-23-82	No		
21011	7	A	7-19-82	Yes	E. cloacae (H) E. coli (H)	
		R	7-29-82	Yes	E. cloacae (M)	
		R	8-13-82	No	Pseudomonas sp. (L)	
		R	9-14-82	No		
		R	11-82	No		
21012	3	A	6-29-82	Yes	E. cloacae (VL)	Group A strep (VL)
		A	7-19-82	Yes	E. cloacae (M) E. coli (M) K. oxytoca (M)	
		R	7-29-82	Yes	E. cloacae (M) E. coli (M)	
		R	8-12-82	Yes	K. oxytoca (M) Pseudomonas sp. (M)	
		R	9-15-82	No		
		R	11-82	No	K. oxytoca (VL)	
Household 403						
40301	43	A	2-8-83	No		
		A	6-10-83	Yes	E. agglomerans (M)	

continued...

TABLE 58. (CONT'D)

Donor ID	Age on 6-30-82	Throat swab category[a]	Specimen collection date	Clinical bacteriology results		
				Abnormal flora?	Gram-negative bacteria (level of growth[b])	Other abnormal flora
40312	6	A	8-17-82	Yes	Achromobacter xylosoxidans (H)	
		A	9-13-82	No		
		C	9-18-82	No		
		C	2-6-83	No		
Household 447						
44702	25	R	9-19-82	Yes	E. agglomerans (M)	
		A	10-7-82	Yes	E. cloacae (H)	
		R	6-8-83	No		
Household 509						
50902	47	A	9-29-82	Yes	S. liquefaciens (M)	
Household 533						
53312	8	A	10-12-82	Yes	E. cloacae (M)	
Household 545						
54502	54	C	1-83	No		
		A	7-19-83	Yes	A. hydrophila (M)	
Household 557						
55701	27	A	9-20-82	Yes	E. cloacae (H)	
55713	10	A	9-20-82	Yes	K. pneumoniae (H)	
55714	5	A	9-20-82	Yes	K. oxytoca (H) Pseudomonas sp. (H)	Group A strep (H)
		A	11-82	No	E. agglomerans (L) Pseudomonas sp. (L)	
		A	12-82	Yes		Group A strep (L)
55715	2	A	9-20-82	Yes	CDC Gr. V E-2 (H)	Group A strep (H)
		A	12-82	Yes		Group A strep (L)

[a] Throat swab categories:
A - acute illness specimen collected while donor was displaying symptoms of a respiratory illness
C - convalescent illness specimen collected within 1 week after recovery from symptoms of the respiratory illness
R - requested throat swab for follow-up or special study

[b] Quantitation of growth on primary culture plates
H: Heavy - growth on three or all quadrants
M: Moderate - growth on first two quadrants
L: Light - growth on first quadrant
VL: Very Light - one to ten colonies on plate

The mother and two sons spent the week of September 5-12 in Houston. The mother reported a sore throat which began on September 12 and ended on September 25; she received antibiotic therapy. Throat swabs were collected from the whole family on September 14 and 15. Heavy levels of E. cloacae were isolated from the throat of the father, but the other family members including the mother were found to have normal throat flora.

Follow-up throat swabs were collected from the mother and sons on November 23. These throat swabs were found to contain normal flora. The father was unavailable at the time that throat swabs were collected and thereafter refused to allow any more swabs to be collected.

Discussion--Enteric Gram-negative bacteria (EGNB), namely E. coli, E. cloacae and K. oxytoca, were repeatedly recovered at moderate or heavy levels throughout the summer from all four members of household 210. EGNB were recovered most regularly and at highest levels from the father (see Table 58). The levels of EGNB recovered from the throat swabs were comparable to those routinely observed with fecal specimens. Isolation of EGNB at these levels in throat swabs may possibly be significant, since these organisms are uncommon in the normal human oropharynx (Youmans et al., 1980). In two separate instances (August 11-13 and September 14-15), all family members who spent the week prior to throat swab collection away from home had normal throat flora, whereas all family members who stayed at home had EGNB throat infections. Clearly, the home environment was associated with the EGNB throat infections. The observed practice of sharing eating utensils may have spread EGNB from one family member to another.

The initial means by which EGNB were introduced into the throats of family members was not clearly established. E. cloacae was recovered at very light levels along with Group A streptococci in the initial June 23 acute illness throat swab from the younger son. The public swimming pool was a possible source, since he developed a respiratory illness attributable to EGNB the same day that he swam there. The evaporative cooler was another possible source, despite failure to recover fecal bacteria from the evaporative cooler reservoir water on August 9. The evaporative cooler hypothesis would explain both the high EGNB recovery rate and levels in the father (due to his habit of sleeping in front of it) and the persistence of EGNB in the throats of all household members while at home during the hottest summer months.

Household 210 was located in the northeastern part of Wilson, approximately 750 m south of the nearest wastewater irrigation rig. All family members received moderate aerosol exposure while at home during the summer 1982 irrigation. Their AEI values were 2.64 for the father, 2.91 for the mother, 2.90 for the older boy and 2.87 for the younger boy. However, the initial recovery of E. cloacae (very light) from the younger boy during a cold which began on June 23 preceded the brief irrigation for aerosol sampling which commenced on July 7. The initial recovery of possibly significant levels of EGNB from the throats of the boys was during illnesses whose onsets on July 13 and July 17 preceded the start on July 21 of the large-scale summer irrigation. Thus, the wastewater aerosol is a very unlikely source of introduction of the EGNB agents compared to the more plausible hypotheses discussed above.

Investigation of Respiratory Illnesses Following Aerosol Exposure: Households 109 and 403, August 1982--

Investigation report--The members of household 403 visited household 109 (located across the road from the eastern edge of the Hancock farm) on the evening of August 8, 1982. It was reported that the visit lasted approximately 2 hours and the children, an 8-year old girl from household 109 and a 6-year old boy from 403, played outside during the visit. It was also reported that irrigation rig 7 which was closest to household 109 was in operation that evening.

On August 9, the girl (10913) reported a sore throat. A culture was taken that day and coxsackievirus B4 was subsequently isolated from her throat swab (see Table 59, footnote e).

A routine stool specimen was collected from the boy (40312) on August 10, 1982 during the regularly scheduled fecal collection. Coxsackievirus B4 was subsequently isolated from that specimen also (see Table 79).

On the evening of August 13 the members of household 403 again visited household 109. The visit lasted approximately 3 hours and the children played outside for the entire visit. (The children rode their bikes along the nearby roads in their outdoor play during one or both visits.) On August 17, the boy reported a sore throat. A throat swab was collected and a heavy level of *Achromobacter xylosoxidans* was isolated from his throat swab (see Table 58).

Assessment of aerosol exposure to causative organisms--The aerosol exposure index values during the summer 1982 irrigation were high for the girl (AEI=11.2) and intermediate for the boy (AEI=2.25), based on the standard exposure estimation methodology and data sources. However, the aerosol exposure of the boy relative to other study participants may have been considerably higher in summer 1982 than AEI=2.25 would indicate. The exposure estimation methodology as applied in 1982 gave virtually no weight to irregular visits to households which were downwind of an operating irrigation rig on the Hancock farm, unless such events also occurred during 1983 when better exposure records were kept (see section 4C). However, better information exists concerning the aerosol exposure of the children in the vicinity of household 109 for the days preceding their illness onsets.

Household 109 was located across the road from an irrigation rig which passed within 120 m of the homestead as it traversed its irrigation circle. This rig sprayed wastewater supplied via pipeline directly from the Lubbock sewage treatment plant on many of the days preceding onset of the illness events. Estimated daily irrigation and aerosol drift patterns from the two nearest rigs were determined for the period from August 1 to August 16. It appears that the girl received substantial exposure to pipeline wastewater aerosol while at home on August 6 and occasional exposure on several other days. However, the daily aerosol drift patterns were approximations, because of limitations in the available data sources: rig operation records did

TABLE 59. OCCURRENCE OF ABNORMAL THROAT FLORA IN ACUTE[a] AND CONVALESCENT[b] ILLNESS THROAT SWABS

		Number (Percent)			
		Clinical bacteriology			
Collection period	Number of illness throat swabs	Group A strep-tococci	Possibly significant bacteria[c]	Probably insignificant bacteria[d]	Clinical virology isolates
ACUTE ILLNESS THROAT SWABS					
1980					
Jul-Sep	3	0 (0)	0 (0)	3 (100)	0
1982					
Jan-Mar	10	0 (0)	0 (0)	0 (0)	0
Apr-Jun	6	1 (17)	0 (0)	1 (16)	0
Jul-Sep	34	8 (24)	10 (29)	2 (6)	1[e] (Cox B4)
Oct-Dec	34	5 (15)	2 (6)	1 (3)	0
1983					
Jan-Mar	22	1 (5)	0 (0)	1 (5)	NA[f]
Apr-Jun	16	5 (31)	1 (6)	2 (13)	NA
Jul-Sep	4	0 (0)	1 (25)	0 (0)	NA
ALL ACUTE	129	20 (15.5)	14 (10.9)	10 (7.8)	1/64 (1.6)
CONVALESCENT ILLNESS THROAT SWABS[g]					
1982					
Jan-Mar	8	0	0	1	0
Apr-Jun	2	0	0	0	0
Jul-Sep	6	0	0	0	0
Oct-Dec	3	1	0	0	0
1983					
Jan-Mar	6	0	0	0	NA
Apr-Jun	8	4	0	0	NA
Jul-Sep	1	0	0	0	NA
ALL CONVALESCENT	34[g]	5 (14.7)	0 (0)	1 (3)	0/15 (0)
ALL ILLNESS TS	163	25 (15.3)	14 (8.6)	11 (6.7)	1/79 (1.3)

[a] Swab obtained while donor was displaying symptoms of a respiratory illness.

[b] Swab obtained within 1 week after donor recovered from symptoms of the respiratory illness.

[c] Enteric Gram-negative bacteria isolated at the moderate or heavy levels.

[d] Enteric Gram-negative bacteria isolated at the light or very light levels and Neisseria spp.

[e] Coxsackievirus B4 isolated from donor 10913 (age 8) in acute throat swab obtained on 8-9-82.

[f] NA - not analyzed. Clinical virology of throat swabs discontinued on 10-23-82.

[g] Includes four illness throat swabs whose illness phase was not reported.

not correlate rig location with hour of the day, yet hourly variation in wind direction frequently was substantial.

Enterovirus levels in the pipeline wastewater were relatively high from August 2 to 10, ranging from 0.06 to 2.2 pfu/mL (see Tables P-3 and P-11 in Appendix P). Virus runs V2 and V3 were performed to monitor pipeline wastewater aerosols on August 2 and 4 respectively during the week preceding the viral isolations of coxsackie B4 from the children. The enterovirus density of the wastewater aerosol sampled on August 4 was extremely high: 16.2 pfu/m^3 on HeLa cells and 18.3 pfu/m^3 on RD cells (primarily poliovirus 1) at 44 m downwind from the irrigation rig (see Table 38). While coxsackievirus B4 was not isolated from the aerosol or wastewater samples in early August 1982 (see Table 39), it was isolated from pipeline wastewater sampled in September 1982 (see Table 25). Due to the high levels of poliovirus in wastewater sampled on August 3 and 4, the detection of a lower level of coxsackievirus B4 could have been masked. Furthermore, although it was not as prevalent as coxsackieviruses B3 and B5, coxsackie B4 was isolated during summer monitoring of Lubbock wastewater in 1980, 1981 and 1983 as well (see Tables P-5 in Appendix P and 26).

Achromobacter xylosoxidans was a prevalent bacterium in both the pipeline and reservoir wastewater during the summer 1982 irrigation. This agent was one of the more frequently isolated bacteria in screens of pipeline and reservoir wastewater samples obtained July 26-27, 1982 (see Table 22).

Discussion--This illness surveillance report documents respiratory illnesses attributable via clinical isolates to coxsackievirus B4 and Achromobacter xylosoxidans, both of which were presumably present in irrigated wastewater. The temporal pattern of wastewater irrigation and illness or agent isolation is consistent with aerosol exposure in this investigation. Assuming an initially low dose of coxsackievirus B4, a minimal incubation period of 24-48 hours would be required to allow multiple cycles of viral replication prior to the onset of clinical symptoms. Exposure of participant 10913 on August 6 and 40312 on August 8 fall within this anticipated time frame. Likewise, colonization of the throat by Achromobacter xylosoxidans to a heavy level would require several days. Thus, the evidence of this illness episode is consistent with the hypothesis that wastewater microorganisms transmitted by wastewater aerosol from spray irrigation infected and produced respiratory illness in the subject children. However, since plausible alternative modes of transmission such as person-to-person spread and contaminated drinking water were not investigated, the evidence for the aerosol exposure hypothesis is inconclusive.

Group A Streptococci

All illness and requested throat swabs were examined for Group A streptococci by the fluorescent antibody technique and also by isolation and identification of β-hemolytic colonies on sheep blood agar. Group A streptococci were isolated from 15.3% (25) of 163 respiratory illness throat swabs as shown in Table 59. The isolation rate of Group A streptococci was about 15% in throat swabs from both the acute and convalescent phases of the illness. Table 58 indicates that Group A streptococci occurrence in respiratory illness throats displayed a seasonal pattern: lowest (1/46=2%) in January-March, highest (10/32=31%) in April-June, and intermediate for the duration of the calendar year (8/48=17% in July-September and 6/37=16% in October-December).

The rate of isolation of Group A streptococci in illness throat swabs was highest (9/24=38%) during April-June 1983. Seven of these specimens were collected on or after May 23 and were presumably unrelated to the spring 1983 irrigation which terminated on April 30, 1983.

The second highest isolation rate of Group A streptococci was 8/40=20% in July-September 1982. Illness throat swabs were collected between July 27 and September 20, 1982 from 26 ill donors whose illness onset may have been between July 21 and September 17, 1982 during the summer 1982 irrigation period. The mean aerosol exposure of the five donors with Group A streptococcal infections (AEI=1.29) was less than the mean AEI of the 21 ill donors who were negative for Group A streptococci (AEI=2.04). Thus, the Group A streptococcal infections which produced respiratory illness during the summer 1982 irrigation appear to have been unrelated to wastewater aerosol exposure.

Enteric Gram-Negative Bacteria (EGNB)

EGNB in Throats--

All illness and requested throat swabs were also plated onto MacConkey agar to detect unusual levels of enteric organisms. Enteric Gram-negative bacteria (EGNB) isolated at the moderate or heavy level in throat swabs were considered to possibly be significant (and were interpreted as an EGNB throat infection), since these organisms are uncommon in the normal human oropharynx, as shown in Table 60 (Youmans et al., 1980).

TABLE 60. MICROORGANISMS FOUND IN THE OROPHARYNX

Microorganisms	Range of prevalence (%)
Staphylococcus aureus	35-40
Staphylococcus epidermidis	30-70
Aerobic corynebacteria (diphtheroids)	50-90
Streptococcus pyogenes (Group A)	0-9
Streptococcus pneumoniae	0-50
Alpha- and nonhemolytic streptococci	25-99
Branhamella catarrhalis	10-97
Neisseria meningitidis	0-15
Haemophilus influenzae	5-20
Haemophilus parainfluenzae	20-30
Gram-negative bacteria, e.g., Klebsiella pneumoniae	Uncommon

Youmans et al., 1980

Various members of the Enterobacteriaceae or Pseudomonas occasionally are found in small numbers from oropharyngeal swabs of healthy humans. However, heavy colonization of the upper respiratory tract by these organisms, as seen in Table 58, at levels similar to those occasionally observed in routine fecal specimens, is a situation that occurs under unusual circumstances.

Data and investigation--EGNB were isolated at the moderate or heavy levels considered possibly significant in 14 (10.9%) of the acute illness throat swabs, but were not found at these levels in any of 34 convalescent illness throat swabs (see Table 59). There was a marked seasonality to the occurrence of these possibly significant isolates in acute illness throat swabs, with 12 occurring between July 19 and October 12, 1982. The other two occurred in June and July 1983. To investigate this phenomenon, bacteriology results were assembled in Table 58 for all throat swabs provided by the 14 donors with EGNB throat infections during the acute phase of a respiratory illness.

The source of all EGNB throat infections in acute illnesses occurring in the study population during the summer of 1982 was pursued. The degree of exposure of throat swab donors with acute illness who had moderate or heavy levels of these bacteria was compared with those who did not (see Table 61). No apparent association was observed with degree of wastewater aerosol exposure or with frequency of eating food prepared at restaurants A or B in Wilson. However, all six of the ill donors with EGNB throat infections lived in homes which used evaporative coolers for air conditioning. The association of EGNB throat infections with evaporative cooler use at home was significant (p=0.02) among the illness throat swab donors. However, since many of the EGNB infected donors were in household 210, the association with evaporative cooler use is not significant (p=0.23) using the household as the unit of observation.

In an additional attempt to characterize this phenomenon, 23 throat swabs were obtained from three groups of healthy adult and teenage participants in mid-September: Hancock farm residents and workers, Wilson residents living at least 800 m from the Hancock farm spray irrigation (Zone 4), and distant rural residents (Zone 5). Surprisingly, EGNB throat infections were about as prevalent in the healthy participants (6/23=26%) in September as they had been in the participants with acute respiratory illness from July to September (8/34=24% from Table 59). Table 62 shows that while the Hancock farm sample had a higher recovery rate (3/7=43%) in the September survey, EGNB were also recovered from the throats of healthy participants in Wilson (1/8) and Zone 5 (2/8). Hence, the phenomenon of moderate and heavy levels of EGNB in the upper respiratory tract appears to have been prevalent throughout the study area, in both ill and healthy participants.

The degree of exposure to potential environmental sources of enteric bacteria of the six healthy throat swab donors surveyed in September 1982 who had EGNB throat infections was compared to the exposure of the 17 who had normal throat flora (see Table 63). Healthy donors with inapparent EGNB throat infections had a higher average aerosol exposure index for summer 1982 than did the healthy donors without EGNB infected throats, but the difference was not statistically significant (p=0.18). The healthy

TABLE 61. INVESTIGATION OF VARIOUS DONOR EXPOSURE VARIABLES FOR ASSOCIATION WITH ENTERIC GRAM-NEGATIVE BACTERIA IN ILLNESS THROAT SWABS IN SUMMER 1982

		Number of illness throat donors by EGNB infection status		
	Period of observation	M or H (infected)	Negative[a] (not infected)	Apparent association
Wastewater Aerosol Exposure	7-19 to 9-20-82			No
Low AEI (<1)		4	6	
Intermediate (1-5)		4	10	
High AEI (>5)		0	1	
Mean AEI		1.5	2.0	
Frequency of Eating at Restaurant A	7-19 to 10-12-82			No
Never		3	9	
1 to 2 times		3	7	
At least once per month		0	2	
Frequency of Eating at Restaurant B	7-19 to 12-7-82			No
Never		6	7	
1 or 2 times		0	7	
At least once per month		0	4	
Use of Evaporative Cooler for Air Conditioning	7-19 to 10-12-82			Yes (p=0.02)[b]
No A/C system		0	5	
Refrigeration A/C		1	16	
Evaporative cooler A/C		5	9	

[a] Includes six donors with very light or light EGNB in illness throat swabs.

[b] One-sided Fisher's exact test, with no A/C and refrigeration A/C rows combined. There is no significant association (p=0.23) using the household as the unit of observation.

TABLE 62. CLINICAL BACTERIOLOGY[a] RESULTS FROM REQUESTED THROAT SWAB SURVEYS OF HEALTHY PARTICIPANTS IN SEPTEMBER 1982 AND JUNE 1983

Group of healthy participants	Throat swabs	Normal flora	Positive for enteric Gram-negative bacteria
FIRST SURVEY: Sep 19-22, 1982			
Hancock farm residents and workers	7	4	3 (43%) - C. diversus-levinea (H), E. aerogenes (H) - E. coli (M) - E. cloacae (H), E. agglomerans (M)
Wilson residents (Zone 4)	8	7	1 (13%) - E. agglomerans (M)
Distant rural residents (Zone 5)	8	6	2 (25%) - E. cloacae (H) - Acinetobacter calcoaceticus var. anitratus (H), K. oxytoca (H)
TOTALS	23	17	6 (26%)
SECOND SURVEY: Jun 6-8, 1983			
Hancock farm residents and workers	6	6	0 (0%)
Wilson residents (Zone 4)	6	6	0 (0%)
Distant rural residents (Zone 5)	7	5	2 (29%) - E. aerogenes (VL) - E. cloacae (VL)
TOTALS	19	17	2 (11%)

[a] Bacteriology only; fluorescent antibody screen not done.

TABLE 63. INVESTIGATION OF VARIOUS DONOR EXPOSURE VARIABLES FOR ASSOCIATION WITH ENTERIC GRAM-NEGATIVE BACTERIA IN REQUESTED THROAT SWAB SURVEY OF HEALTHY DONORS IN SEPTEMBER 1982

	Number of healthy throat swab donors by EGNB infection status		Apparent association (p-value)
	M or H (infected)	Negative (not infected)	
Wastewater Aerosol Exposure (in summer 1982)			No Insufficient data (?)
Low AEI (<1)	2	7	
Intermediate (1-5)	1	6	
High AEI (>5)	3	4	
Mean AEI	35.8	6.9	
Geometric mean AEI	3.64	1.17	No ($p=0.18$)[a]
Frequency of Eating at Restaurant A (in summer 1982)			No ($p=0.14$)[b] Insufficient data (?)
Seldom or never	2	8	
At least once per month	4	3	
Frequency of Eating at Restaurant B (in summer 1982)			No
Never	4	9	
At least once per month	2	2	
Use of Evaporative Cooler for Air Conditioning (A/C)			No ($p=0.22$)[b]
Refrigeration or no A/C	2	9	
Evaporative cooler A/C	4	6	
Contaminated Private Drinking Water Well (in June 1982 and/or Nov/Dec 1982)			Insufficient data
Acceptable	2	2	
Contaminated[c]	2	1	

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[a] One-sided t-test of difference in means in two independent populations; ln(AEI) transformation used to reduce variance inequality.

[b] One-sided Fisher's exact test.

[c] Total coliforms, fecal coliforms, or fecal streptococci ≥ 1 cfu/100 mL.

donors with EGNB throat infections also tended to eat at restaurant A more often, but this difference also was not significant (p=0.14). The donors with inapparent EGNB throat infections were more likely to reside in a household using an evaporative cooler for air conditioning, but again there was not a significant association (p=0.22). Because of the small sample sizes, none of these three exposure variables nor contaminated private drinking water wells can be ruled out as possible risk factors. The frequency of eating at restaurant B was not a risk factor.

A second throat swab survey of 19 healthy donors was performed in June 1983. None of them had throat infections with moderate or heavy levels of EGNB (see Table 62), although two of the distant rural participants had very light (probably insignificant) levels of these bacteria in their throats. Thus, the prevalence of EGNB throat infections in the acute upper respiratory illness population reflected the prevalence in the healthy population during each survey. Respiratory ill and well participants both had an EGNB throat infection prevalence above 25% in September 1982 and both had a lower prevalence of these bacterial infections (approximately 10% in the illness population and below 5% in the healthy population) in June 1983.

Discussion--The remarkable aspect of the results of illness specimen throat swabs of some LISS participants during July to October 1982 (Table 58) is not the mere presence of Gram-negative enterics, but the unusually high levels of the organisms. EGNB had been observed occasionally before and after these dates at the VL or L level, but seldom at the M or H levels. The oropharynx of healthy humans is not commonly assumed to be an environment favoring growth or persistence of EGNB. For example, one study (Johanson et al., 1969) examined the oropharyngeal flora (presence/absence only) of five groups of adult subjects. Only 2% of normal subjects, whether hospital or nonhospital associated, and 0-2% of patients on the psychiatry service yielded throat cultures positive for EGNB. However, the levels of positive cultures in a single culture survey of moderately ill and moribund patients was 16% and 57%, respectively. Other evidence suggests that increased oropharyngeal colonization by EGNB may be associated with upper respiratory illness (URI). In a study carried out in a Puerto Rican hospital (Ramirez-Rhonda et al., 1980), presence of EGNB was found in the oropharynx of 14% of normal adult outpatients. Colonization of the oropharynx of hospital staff with EGNB ranged from 12 to 18% in the absence of illness, but increased to 38 to 60% in individuals with URI, presumably of viral origin. K. pneumoniae was the most frequent isolate, followed by E. coli and Enterobacter spp.

Although high levels of EGNB were observed in acute illness throat swabs of LISS participants, they were largely confined to specimens obtained in the summer months, which would tend to argue against an association with URI of other etiology, particularly viral. Also, high levels of EGNB were observed in requested throat cultures of a similar proportion of healthy LISS participants during the same period.

Oropharyngeal EGNB levels appear to have been much higher in the infected LISS participants than in infected subjects in the Puerto Rican study. Ramirez-Rhonda et al. (1980) determined the total numbers of EGNB/mL of oropharyngeal fluid of hospital staff with URI (151 subjects). The levels of EGNB/mL in positive individuals were <10 cfu (9%), 10 to 100 cfu (54%), 100 to 300 cfu (38%), and >300 cfu (1%). For LISS participants with high levels of oropharyngeal EGNB, it would appear from quality assurance studies (see Table A-34 in Appendix A) that isolation at the M or H level would require $>10^5$ to 10^7 cfu/mL of the organisms. Such numbers would be inconsistent with all but the 1% of subjects with URI in the study of Ramirez-Rhonda et al. (1980) who may have had comparable levels of organisms (i.e., the >300 cfu/mL group).

Use of antibiotics could conceivably reduce susceptible components of the normal flora that would normally prevent colonization of the oropharynx by EGNB through bacterial interference. For example, pharyngeal colonization with α-hemolytic streptococci, the most prevalent group of organisms observed on throat cultures, appears to protect neonates in a hospital environment from pharyngeal colonization with EGNB (Goldmann, 1981). However, the seasonal incidence of high levels of EGNB in LISS participants and their isolation from healthy subjects would argue against this interpretation. Also, the role of antibiotics as a predisposing factor for colonization of the oropharynx by EGNB is a subject of some controversey, since there have been studies in which use of antibiotics was (Haverkorn and Michel, 1979) and was not (Johanson et al., 1969, 1972) correlated.

The factors (perhaps use of evaporative coolers) responsible for the high levels of EGNB in LISS participants remain unresolved. The studies of Philpot and MacDonald (1980) suggested that pharyngeal carriage rates of EGNB may differ substantially between different groups of normal individuals and challenged the common assumption that a high rate of carriage of the organisms exclusively is associated with hospitalization or debility. The prevalence of all EGNB (presence/absence) recovered from throat swabs of healthy Australian adults (31 subjects), Malaysian adults (25 subjects), and Malaysian children (25 subjects) were 9%, 36% and 4%, respectively. The prevalence of the organisms in Malaysian adults (28% of 25 subjects) and children (12% of 25 subjects) with sore throats was not markedly different from that observed for the healthy counterparts. It is interesting and perhaps relevant to the LISS EGNB throat data that the investigators noted that ''in each case the numbers of these bacteria detected were not great.'' They suggested that the higher carriage rate in Malaysian as opposed to Australian adults might be due to ''food preferences or other social habits.''

Abnormal fecal levels (AFL) of selected EGNB--

Clinical bacteriologic analysis (see Figure 14) was performed on 34 gastrointestinal and respiratory illness stool specimens. The results are presented in Table 64. Normal fecal flora were absent or present at abnormally low levels in 8 (24%) of these illness stools, especially in convalescent specimens. This probably indicates antibiotic therapy, but may reflect problems with sample processing or shipping. AFL of selected EGNB were observed at the moderate or heavy level in 5 (15%) of these illness fecal specimens. Occurrence of AFL of EGNB was higher in the illness fecal specimens of adults age 18-44 (50%) than in children or older adults (see Table 65). All five isolates were _Klebsiella_ _pneumoniae_ or _K_. _oxytoca_. The M or H _Klebsiella_ levels were from illness fecal specimens collected

TABLE 64. OCCURRENCE OF ABNORMAL LEVELS OR FLORA IN ACUTE[a] AND CONVALESCENT[b] ILLNESS FECAL SPECIMENS

	Number (Percent)				
		Clinical bacteriology		Viruses	
	Number of illness fecal specimens	Absence or decrease of normal fecal flora	Possibly significant bacteria[c]	Clinical virology isolates	Electron microscopy detections
1982					
Jan-Mar	1	0	0	0	0
Apr-Jun	4[d]	2 (50)	1 (25)	0	1[d] (33)
Jul-Sep	3	1 (33)	0	2 (67)	0
Oct-Dec	2	0	0	NA[d]	2 (100)
1983					
Jan-Mar	12	3 (25)	0	1 (8)	1 (8)
Apr-Jun	11	1 (9)	4 (36)	2 (18)	0
Jul-Sep	1	1 (100)	0	1 (100)	0
All acute	10	1 (10)	0	2[d] (25)	4 (40)
All convalescent	8	4 (50)	1 (13)	1 (13)	0
Illness phase not reported	16	3 (19)	4 (25)	3 (19)	0
TOTAL	34	8 (24)	5[c] (15)	6/32[d] (19)	4/33[d] (12)

[a] Specimen obtained while donor was displaying symptoms of a gastrointestinal or respiratory illness.

[b] Specimen obtained within 1 week after donor recovered from symptoms of the gastrointestinal or respiratory illness.

[c] Enteric Gram-negative bacteria isolated at the moderate or heavy level. All isolates were Klebsiella (pneumoniae or oxytoca).

[d] Illness fecal specimens not analyzed (NA): 2 by tissue culture virology and 1 by EM.

TABLE 65. AGE-SPECIFIC DISTRIBUTION OF ABNORMAL LEVELS OR FLORA IN ILLNESS FECAL SPECIMENS

Donor age on 6-30-82, years	No. positive/No. specimens analyzed (percent)		
	Klebsiella at M or H level	Viruses: Isolated by cell culture	Viruses: Detections by EM
0-5	1/9 (11)	3/8 (38)	3/9 (33)
6-17	0/9 (0)	2/8 (25)	1/9 (11)
18-44	4/8 (50)	0/8 (0)	0/7 (0)
45-64	0/5 (0)	0/5 (0)	0/5 (0)
65+	0/3 (0)	1/3 (33)	0/3 (0)
All ages	5/34 (15)	6/32 (19)	4/33 (12)

on June 24, 1982, May 17, 1983, and three on June 1, 1983. Each of these illness onsets followed termination of the spring irrigation period and preceded the start of the summer irrigation. The prevalence of moderate or heavy Klebsiella was 33% in 15 illness fecal specimens from May and June.

Surprisingly, the occurrence of moderate or heavy levels of EGNB was not much higher (15%) in fecal specimens collected during gastrointestinal and respiratory illness than in throat swabs collected during the acute phase of respiratory illness (11%). In addition, the seasonal pattern of occurrence was somewhat different: moderate or heavy levels of these EGNB were only found in illness stools during May and June, whereas they were most prevalent in acute illness throat swabs from July to early October.

Some of the preceding discussion concerning factors influencing colonization of the oropharynx by EGNB also is applicable to AFL of selected EGNB. However, an important difference is that organisms such as Klebsiella, Enterobacter, and Citrobacter along with the almost ubiquitous E. coli are common and significant components of the facultatively anaerobic normal flora of the gut (Lennette et al., 1980), in contrast to the rarer occurrence of the organisms in smaller numbers in the oropharynx. Various members of the family Enterobacteriaceae, aside from the overt pathogens Salmonella, Shigella and Yersinia, are commonly encountered as pathogens only in special circumstances (e.g., as the major causes of nosocomial infections). However, toxin-producing E. coli are a common cause of diarrhea in normal subjects and other toxin-producing coliforms may at times be associated with acute diarrhea. In addition, increased prevalence and levels of intestinal colonization by organisms such as Klebsiella in a hospital environment have been associated with illness, duration of hospitalization, and use of antibiotics (Haverkorn and Michel, 1979; Goldmann et al., 1978; Selden, et al., 1971).

Viruses

Viruses in Illness Throat Swabs--

Illness throat swabs were examined for viruses by tissue culture techniques as diagrammed in Figure 16. As Table 59 illustrates, viruses were rarely isolated from illness throat swabs; a single viral isolate was recovered from 79 specimens (1.3%). Coxsackievirus B4 was isolated in a throat swab collected on August 9, 1982 from 10913 while she had a sore throat. The circumstances are thoroughly discussed in the respiratory illness investigation above, since it may have been associated with wastewater aerosol exposure. Because of the low viral recovery rate, clinical virologic analysis of illness throat swabs was discontinued in October 1982.

Viruses in Illness Fecal Specimens--

Fecal specimens collected during gastrointestinal and respiratory illnesses were examined for viruses both by tissue culture techniques and by electron microscopy (EM). Viral prevalence is summarized in Table 64.

Viral isolates were recovered from 6 (19%) of 32 illness fecal specimens analyzed by tissue culture. The recovery rates from acute and convalescent phase specimens were similar. Viral recovery showed a seasonal pattern: markedly higher for July-September (3/4=75%) than in earlier calendar quarters (8% for January-March and 13% for April-June). Three of the viral isolates were identified by fluorescent staining as adenoviruses, but the other three could not be identified by enterovirus typing pools or fluorescent staining (see Table 66). Viral recovery appears to show an age-related pattern (see Table 65), with higher recovery rates from children and the elderly.

Illness onset associated with three of the viral isolates (two from illness fecal specimens and one from an illness throat swab) occurred during the summer 1982 irrigation period. Both of the ill children with fecal isolates received an intermediate level of aerosol exposure (see Table 66). Only one donor provided a negative illness fecal specimen during the summer irrigation. These data are insufficient to address the question of possible association of the illness viral isolates with wastewater aerosol exposure.

Virus-like particles were detected in 4 (12%) of the 33 illness fecal specimens examined by EM (see Table 64). All of the detected virus-like particles were in acute illness specimens (40% detection rate). The detection of virus-like particles was strongly associated with illness specimens from young children (see Table 65), with a positive rate of 33% in ill donors of age 0-5. The types of virus-like particles detected by EM are presented in Table 66 and Figure 27.

Norwalk-like particles were detected in an acute illness specimen from one boy (21112) in May 1982. This specimen, a simultaneous specimen from his older sister (21111), and four pairs of sera were sent to Dr. N. R. Blacklow's laboratory at the University of Massachusetts for examination by RIA. Both stools were negative for Norwalk antigen and no seroconversions to Norwalk virus were detected.

TABLE 66. IDENTIFICATION AND COMPARISON OF VIRAL ISOLATES BY CELL CULTURE AND VIRUS-LIKE PARTICLES BY EM IN ILLNESS FECAL SPECIMENS

Collection date	Donor ID	Age on 6-30-82	Illness phase[a]	Decrease in normal fecal flora?[b]	Clinical virology agent isolated	Virus-like particles detected by EM	Donor AEI, (if onset during irrigation)
5-18-82	21112	1	A	Yes	(none)	Norwalk virus-like	
8-4-82	60111	0	A	No	Adenovirus	(none)	1.70
9-24-82	40312	6	C	Yes	Unidentified virus	(none)	2.25[c]
11-16-82	20211	13	A	No	(not analyzed)	Astrovirus-like	
12-6-82	21112	1	A	No	(not analyzed)	Calicivirus-like	
2-14-83	51013	0	A	No	Adenovirus	Adenovirus-like	
5-17-83	53101	74	?	No	Unidentified virus	(none)	
6-1-83	60111	0	?	No	Adenovirus	(none)	
9-8-83	21111	8	?	Yes	Unidentified virus	(none)	2.85

[a] Illness phase: A - acute, C - convalescent, ? - not reported.

[b] A decrease in normal fecal flora probably indicates antibiotic therapy, but may reflect problems with sample processing or shipping.

[c] AEI value may underestimate aerosol exposure (see Illness Investigation involving household 403).

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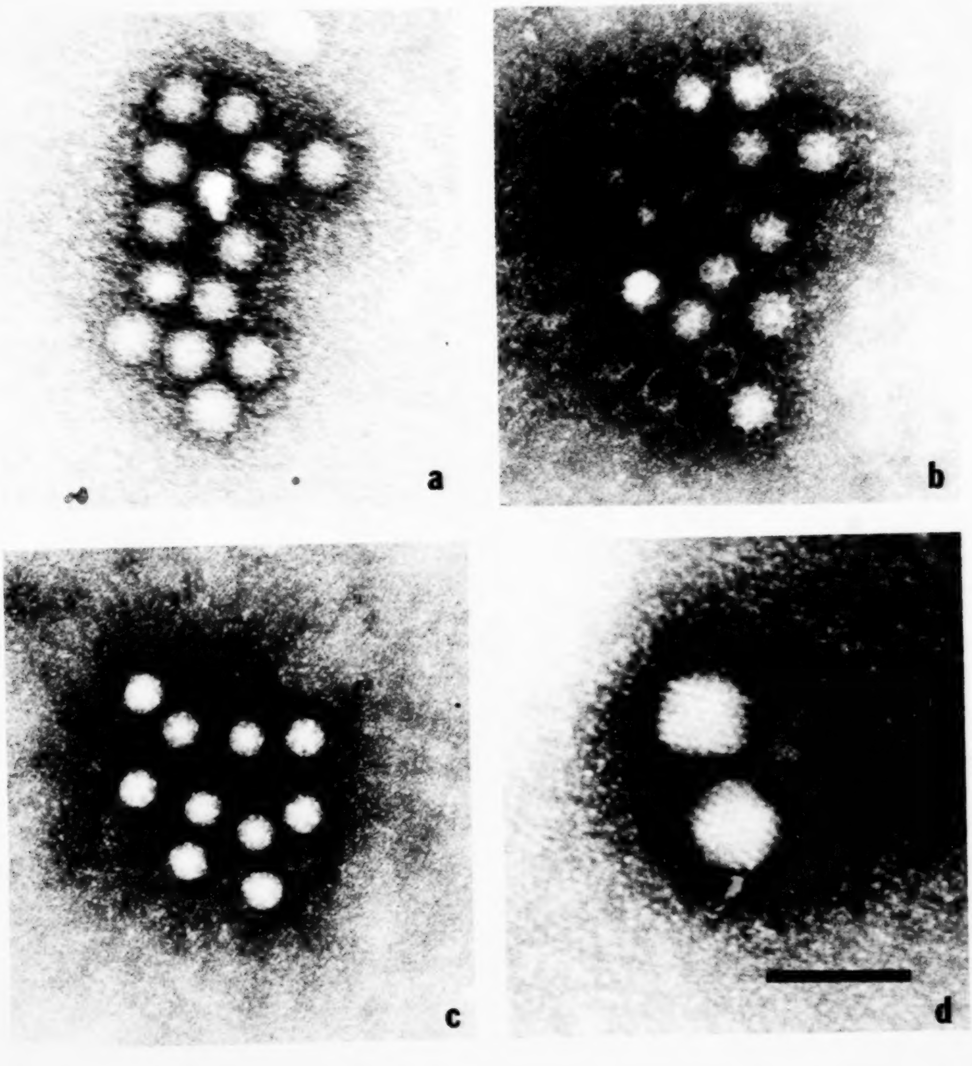

Figure 27. Virus particles observed by EM in illness stool specimens.
(a) Norwalk-like particles in the first illness stool (5-82) of 21112.
(b) Calicivirus-like particles in the second illness stool (12-82) of 21112.
(c) Astrovirus-like particles in the stool of 20211 (11-82).
(d) Adenovirus-like particles in the stool of 51013 (2-83).
Bar = 100 nm for a-d.

Calicivirus-like particles were detected in a second illness specimen from the same boy in December 1982. Astrovirus-like particles were detected in a November 1982 illness specimen from another girl (20211). Requested stools received in January 1983 from these children were negative for virus-like particles.

As shown in Table 66, adenovirus-like particles were detected by EM in one of the three illness fecal specimens from which an adenovirus was isolated by tissue culture. This 33% adenovirus detection rate by EM in adenovirus-positive specimens is similar to the 40% detection rate of coronavirus-like particles by EM in routine specimens previously found to be positive (see EM Quality Assurance).

The onset of each of the four illnesses for which EM analysis detected virus-like particles was during times when there was no sustained wastewater irrigation. Thus, these EM-detected viral infections presumably were unrelated to wastewater irrigation operations.

Of the enteric viruses frequently associated with gastroenteritis, only human rotaviruses have been reproducibly cultivated outside the human host. Therefore, their involvement in diarrheal illness is far from certain. To date, only rotaviruses and Norwalk virsus are recognized as medically important agents of human gastroenteritis. Recently, enteric adenoviruses have been recognized for their possible role in diarrheal illness (Cukor and Blacklow, 1984).

While astroviruses are found in stool specimens obtained from cases of intestinal illness, experimental ingestion of astrovirus-containing fecal filtrates by nine volunteers resulted in viral shedding by only two individuals, neither of whom developed diarrhea or vomiting (Kurtz et al., 1979). In a prospective study involving 447 children hospitalized with infectious gastroenteritis, Ellis and associates (1984) found no significant association of astrovirus with this disease when compared to childred treated for respiratory infections. Conversely, rotavirus ($p<0.0001$), adenovirus ($p<0.01$) and calicivirus ($p<0.01$) were associated with diarrheal illness in young children.

G. CLINICAL BACTERIOLOGY OF ROUTINE FECAL SPECIMENS

Summary Data

Routine fecal specimens provided by donors in scheduled collection weeks were analyzed for bacteria using procedures summarized in Figure 14. In all cases, the organisms isolated were reported as a function of the level of growth (very light to heavy) observed on primary plating media.

Results from 268 specimens collected during 1980 and 1981 are presented in Table 67. Approximately 90% of these baseline specimens were obtained from children age 12 or less. Beginning in January 1982 one randomly selected adult from each household was also asked to donote specimens. The results from 725 specimens collected in 1982 and from 517 specimens collected in 1983 are shown in Tables 68 and 69, respectively.

TABLE 67. ORGANISMS ISOLATED FROM ROUTINE FECAL SPECIMENS DURING 1980 AND 1981
(268 Specimens)[a]

	Quantitation of growth[b] [percent (number) positive]				
Organism	Heavy	Moderate	Light	Very light	Total[c]
Aeromonas hydrophila	-	-	0.4 (1)	0.4 (1)	0.7 (2)
Candida albicans[d]	-	0.9 (2)	7.2 (15)	12.9 (27)	21.5 (45)
Citrobacter diversus	-	-	-	0.4 (1)	0.4 (1)
Citrobacter freundii	0.7 (2)	2.6 (7)	3.4 (9)	4.5 (12)	11.2 (30)
Citrobacter spp.	-	-	-	0.4 (1)	0.4 (1)
Enterobacter aerogenes	-	0.4 (1)	0.7 (2)	0.7 (2)	1.9 (5)
Enterobacter cloacae	0.4 (1)	2.6 (7)	4.1 (11)	4.5 (12)	11.6 (31)
Enterobacter sakazakii	-	0.4 (1)	0.7 (2)	1.1 (3)	2.6 (7)
Escherichia coli	40.7 (109)	44.8 (120)	11.9 (32)	1.9 (5)	99.6 (267)
Hafnia alvei	-	-	0.7 (2)	0.7 (2)	1.5 (4)
Klebsiella oxytoca	-	0.7 (2)	4.9 (13)	2.6 (7)	8.2 (22)
Klebsiella pneumoniae	1.1 (3)	2.6 (7)	9.3 (25)	9.3 (25)	22.4 (60)
Klebsiella spp.	-	-	0.7 (2)	-	0.7 (2)
Morganella morganii	-	-	0.7 (2)	0.7 (2)	1.5 (4)
Proteus mirabilis	-	-	0.4 (1)	-	0.4 (1)
Providencia alcalifaciens	-	0.4 (1)	0.4 (1)	0.7 (2)	1.5 (4)
Fluorescent Pseudomonas gr.	-	-	1.5 (4)	2.6 (7)	4.1 (11)
Pseudomonas spp.	-	-	0.4 (1)	-	0.4 (1)
Serratia liquefaciens	-	-	0.4 (1)	-	0.4 (1)
Serratis odorifera	-	-	0.7 (2)	-	0.7 (2)
Staphylococcus aureus	0.4 (1)	2.2 (6)	23.9 (64)	8.2 (22)	34.7 (93)
Staphylococcus epidermidis	-	-	0.4 (1)	1.1 (3)	1.5 (4)
Yersinia enterocolitica	-	0.4 (1)	-	-	1.1 (3)

[a] From Data Collection Periods 015, 017, 019, 108, 110, 112, 114, 117 and 118.

[b] Quantitation of growth on primary culture plates
Heavy - growth on three or all guadrants
Moderate - growth on first two quadrants
Light - growth on first quadrant
Very Light - one to ten colonies on plate

[c] Includes positives by enrichment only

[d] Based on 209 specimens (procedures for isolation of C. albicans began in Data Collection Period 019)

TABLE 68. ORGANISMS ISOLATED FROM ROUTINE FECAL SPECIMENS DURING 1982
(725 Specimens)[a]

Organism	Quantitation of growth[b] [percent (number) positive]				
	Heavy	Moderate	Light	Very light	Total[c]
API Group I	–	–	–	0.1 (1)	0.3 (2)
Aeromonas hydrophila	–	–	0.1 (1)	0.1 (1)	0.3 (2)
Candida albicans	0.1 (1)	1.0 (7)	3.0 (22)	10.2 (74)	14.3 (104)
Chromobacterium	–	0.1 (1)	0.4 (3)	–	0.7 (5)
Citrobacter amalonaticus	–	–	0.1 (1)	–	0.1 (1)
Citrobacter diversus-levinea	–	0.7 (5)	0.1 (1)	–	0.8 (6)
Citrobacter freundii	–	1.1 (8)	1.2 (9)	1.1 (8)	3.4 (25)
Citrobacter spp.	–	–	0.1 (1)	0.1 (1)	0.3 (2)
Enterobacter aerogenes	0.7 (5)	0.8 (6)	1.7 (12)	0.4 (3)	3.7 (27)
Enterobacter agglomerans	0.3 (2)	–	0.3 (2)	0.1 (1)	0.7 (5)
Enterobacter cloacae	1.8 (13)	3.2 (23)	4.0 (29)	3.0 (22)	12.4 (90)
Enterobacter sakazakii	–	0.1 (1)	0.7 (5)	–	0.8 (6)
Enterobacter spp.	–	–	–	0.1 (1)	0.1 (1)
Escherichia coli	36.7 (266)	44.1 (320)	13.9 (101)	3.0 (22)	98.6 (715)
Hafnia alvei	0.1 (1)	0.1 (1)	0.1 (1)	0.1 (1)	0.6 (4)
Klebsiella oxytoca	0.1 (1)	1.8 (13)	3.4 (25)	1.2 (9)	7.3 (53)
Klebsiella pneumoniae	4.6 (33)	7.3 (53)	8.1 (59)	3.3 (24)	25.5 (185)
Morganella morganii	–	–	0.3 (2)	0.4 (3)	0.7 (5)
Proteus mirabilis	–	0.6 (4)	0.3 (2)	0.3 (2)	2.2 (16)
Proteus rettgeri	–	0.1 (1)	–	0.1 (1)	0.4 (3)
Proteus vulgaris	–	–	0.3 (2)	0.1 (1)	0.4 (3)
Providencia alcalifaciens	–	0.1 (1)	–	0.1 (1)	0.3 (2)
Fluorescent Pseudomonas gr.	0.1 (1)	1.2 (9)	1.7 (12)	1.7 (12)	5.2 (38)
Pseudomonas aeruginosa	–	–	0.6 (4)	–	0.7 (5)
Pseudomonas spp.	–	–	0.1 (1)	–	0.1 (1)
Salmonella spp.	0.1 (1)	–	–	–	0.1 (1)
Serratia fonticola	0.1 (1)	–	–	–	0.1 (1)
Serratia marcescens	–	–	0.3 (2)	–	0.3 (2)
Serratia odorifera	–	–	0.1 (1)	–	0.1 (1)
Staphylococcus aureus	–	2.1 (15)	6.3 (46)	6.5 (47)	14.9 (108)

a From Data Collection Periods 201, 205, 207, 212, 216 and 219

b Quantitation of growth on primary culture plates

Heavy – growth on three or all guadrants
Moderate – growth on first two quadrants
Light – growth on first quadrant
Very Light – one to ten colonies on plate

c Includes positives by enrichment only

TABLE 69. ORGANISMS ISOLATED FROM ROUTINE FECAL SPECIMENS DURING 1983
(517 Specimens)[a]

Organism	Quantitation of growth[b] [percent (number) positive]				
	Heavy	Moderate	Light	Very light	Total[c]
API Group I	0.2 (1)	0.2 (1)	0.2 (1)	0.4 (2)	1.0 (5)
Aeromonas hydrophila	0.2 (1)	0.4 (2)	0.2 (1)	-	0.8 (4)
Candida albicans	-	0.6 (3)	6.4 (33)	9.1 (47)	16.1 (83)
Chromobacterium	-	0.2 (1)	-	0.2 (1)	0.4 (2)
Citrobacter amalonaticus	-	0.2 (1)	0.2 (1)	-	0.4 (2)
Citrobacter diversus-levinea	0.4 (2)	-	-	-	0.4 (2)
Citrobacter freundii	0.2 (1)	0.4 (2)	0.8 (4)	1.0 (5)	2.5 (13)
Enterobacter aerogenes	0.6 (3)	1.7 (9)	1.5 (8)	-	3.9 (20)
Enterobacter agglomerans	0.2 (1)	0.2 (1)	0.2 (1)	-	0.8 (4)
Enterobacter cloacae	1.5 (8)	4.6 (24)	4.6 (24)	1.5 (8)	12.8 (66)
Enterobacter sakazakii	0.4 (2)	0.4 (2)	1.2 (6)	0.4 (2)	2.3 (12)
Escherichia coli	50.1 (259)	35.8 (185)	8.3 (43)	1.7 (9)	96.7 (500)
Hafnia alvei	-	-	0.4 (2)	0.2 (1)	0.6 (3)
Klebsiella oxytoca	0.8 (4)	1.7 (9)	0.8 (4)	0.8 (4)	4.3 (22)
Klebsiella ozaenae	-	-	-	0.2 (1)	0.2 (1)
Klebsiella pneumoniae	4.1 (21)	9.3 (48)	8.1 (42)	2.7 (14)	24.4 (126)
Moraxella spp.	-	0.4 (2)	0.2 (1)	0.2 (1)	1.0 (5)
Morganella morganii	0.2 (1)	-	-	-	0.2 (1)
Plesiomonas shigelloides		2 by enrichment only			0.4 (2)
Proteus mirabilis	0.2 (1)	0.4 (2)	0.4 (2)	-	1.2 (6)
Proteus rettgeri	-	0.4 (2)	-	-	0.4 (2)
Pseudomonas aeruginosa	-	0.2 (1)	0.2 (1)	0.2 (1)	0.8 (4)
Pseudomonas spp.	-	0.6 (3)	0.4 (2)	-	1.4 (7)
Serratia liquefaciens	0.2 (1)	-	0.2 (1)	-	0.4 (2)
Serratia odorifera	-	-	-	0.2 (1)	0.2 (1)
Staphylococcus aureus	0.6 (3)	2.1 (11)	8.1 (42)	4.8 (25)	15.7 (81)

a From Data Collection Periods 303, 308, 312, 315 and 317

b Quantitation of growth on primary culture plates

Heavy - growth on three or all guadrants
Moderate - growth on first two quadrants
Light - growth on first quadrant
Very Light - one to ten colonies on plate

c Includes positives by enrichment only

Bacterial Infection Events

Infection events for bacterial agents have been defined in Section 4G. An infection event is not equated with disease, the latter being indicated by detectable alterations in normal tissue functions (i.e., clinical manifestations of illness). Infection is used in the broader sense of the entrance and multiplication of a microbe in the body.

Specimens which failed to yield any growth, or which yielded organisms by enrichment only, were excluded from the data set in defining bacterial infections and infection events. The lack of organisms, in these cases, is likely to have been due to problems with sample processing, shipping or use of antibiotics by participants.

The densities of the overt and opportunistic pathogens in bacterial infection event Categories 1-3 and of indicator bacteria in the sprayed pipeline and reservoir wastewater were monitored regularly. The environmental data previously presented indicate that the overt and opportunistic pathogens, except *Shigella*, were present periodically. *Aeromonas hydrophila*, the fluorescent *Pseudomonas* group, and *Klebsiella* consistently were prominent organisms in the wastewater. Pipeline wastewater always had much higher microorganism levels than reservoir wastewater.

Infections by Overt Pathogens

The results for Category 1 organisms (overt enteric bacterial pathogens) are presented in Table 70.

TABLE 70. INFECTIONS BY OVERT ENTERIC BACTERIAL PATHOGENS (CATEGORY 1)

	Baseline Period[a]	Irrigation Period[b]
Fecal specimens	369	1,091
Infections by major enteric bacteria	3[c] (1%)	1[d] (0.1%)

[a] Fecal collection periods from June 1980 through January 1982.

[b] Fecal collection periods from March 1982 through August 1983.

[c] Three Y. enterocolitica, two by enrichment only: June-July 1981.

[d] Salmonella Group C_1, heavy level, June 1982.

No major bacterial enteric pathogens were isolated from the direct platings of the 369 routine fecal specimens collected during the baseline preirrigation periods. However, *Y. enterocolitica* was isolated after enrichment from three different individuals in June and July 1981. Likewise, the analysis of 1,091 routine fecal specimens collected from participants after commencement of spray irrigation failed to reveal major bacterial enteric pathogens, except for the isolation of a serologically confirmed Salmonella group C_1. The organism was isolated at the heavy level from an adult male in June 1982. Subsequent requested fecal specimens also yielded this organism from the same individual and his son (see Illness Investigations in Section 5F). Because so few infections by overt enteric bacterial pathogens were observed from routine fecal samples during the preirrigation and irrigation periods, the data were not subjected to futher analysis.

The overt enteric pathogens are of major clinical significance because they often are associated with disease and even inapparent or subclinical infections may provide a source for infection and disease in others. In spite of a rigorous search for overt enteric bacterial pathogens, the number of isolations from the routine fecal specimens was small in baseline monitoring (three) and periods after commencing of irrigation (one). Overt pathogens often were detected in the wastewater sampling with the exception of Shigella, which may have been below the level of detection by the direct plating and enrichment procedures used. The size of inoculum required to produce disease in humans varies widely for enteric pathogens (Gangarosa, 1978), ranging, for example, from as few as 10 organisms for Shigella to 10^8 for most serotypes of Salmonella. Thus, while most of the major enteric bacterial pathogens were present in the sprayed wastewater, the reduced rate of infections by these pathogens after irrigation commenced indicates that no increased risk of these infections was associated with exposure to wastewater.

Klebsiella Infections

A single genus, Klebsiella, produced most of the observed infections by the possibly significant opportunistic bacterial pathogens (Category 2). Since more definitive risk factors and etiology might be identified for a more specific group of organisms, the Klebsiella infections were analyzed separately from the infections by the other opportunistic pathogens.

Klebsiella pneumoniae was the agent recovered in 91% of the Klebsiella infections. The remaining infections were due to K. oxytoca.

The prevalence of Klebsiella infections is presented in Table 71. Although they were infrequent during the baseline period, Klebsiella infections occurred throughout 1982 and 1983 and were especially prevalent during both of these summers.

An exploratory analysis was conducted to identify possible risk factors for Klebsiella infections. During the time interval from January 1982 through August 1983 when most of the Klebsiella infections were observed, donors having Klebsiella infections were compared to the donors who were not infected with regard to demographic, socioeconomic, lifestyle, drinking water and health history characteristics. The association of Klebsiella infection status (infected at least once vs. never infected) with each characteristic was evaluated by a chi-square test using Cochran's cell size rule and Yates' continuity correction for 2x2 tables. When a difference was observed at $p<0.05$, the characteristic was considered a possible risk factor.

TABLE 71. PREVALENCE OF BACTERIAL INFECTIONS BY COLLECTION MONTH

Specimen collection month	Routine fecal donors	Prevalence rate (Infections per 100 donors)		
		Opportunistic pathogens		Bacteria prominent in wastewater
		Klebsiella	Others	
1980				
Jul	22	0	0	0
Aug	36	5.6	0	0
Sep	47	0	0	0
1981				
Apr/May	27	0	3.7	0
Jun	44	2.3	0	0
Jul	29	0	0	0
Aug/Sep	35	0	5.7	0
1982				
Jan	105	1.0	0	1.0
Mar	125	1.6	0	0.8
Mar/Apr	118	0.8	0	1.7
Jun	124	8.1	0	0.8
Aug	107	10.3	1.9	1.9
Sep	110	8.2	0	2.7
1983				
Feb	97	4.1	1.0	5.2
Apr	107	1.9	4.7	3.7
Jun	100	3.0	0	0
Jul	103	4.9	1.9	8.7
Aug	101	9.9	2.0	3.0

In contrasting the 37 fecal donors having *Klebsiella* infections in 1982 and 1983 with the 71 donors not experiencing *Klebsiella* infections during the same period of observation, gender was the only factor which appeared to be significantly associated with the infected donors (see Table 72). Whereas 34% of all donors had *Klebsiella* infections, 44% of the female donors experienced *Klebsiella* infections, which is a nominally significant association at the p=0.02 level. This excess of *Klebsiella* infections among female donors relative to male donors occurred at all age levels. An equally high proportion (i.e., 50%) of males aged 65 and above had *Klebsiella* infections, but this association was only of borderline significance (p=0.07). Repeated *Klebsiella* infections were observed over intervals ranging up to 20 months in 12 donors, 10 of whom were females. Hence being female appears to be a risk factor for infection by *Klebsiella* in the population studied.

The clinical significance of *Klebsiella* infection was also investigated. The incidence densities of self-reported respiratory, gastrointestinal, and skin illnesses in the 2-week periods prior, concurrent and subsequent to the fecal collection were compared for all routine fecal specimens with heavy *Klebsiella* growth (i.e., ''infected''), with moderate *Klebsiella*

TABLE 72. EXPLORATORY ANALYSIS OF THE ASSOCIATION OF INDIVIDUAL CHARACTERISTICS WITH BACTERIAL INFECTION PREVALENCE

	Klebsiella	Other Opportunistic pathogens	Bacteria prominent in wastewater
Period of observation	Jan-Sep 1982 Feb-Aug 1983	Apr-Sep 1981 Aug-Sep 1982 Feb-Aug 1983	Jan-Sep 1982 Feb-Aug 1983
Donors infected	37 (34%)	14 (16%)	19 (18%)
Donors not infected[a]	71	72	85
Characteristics associated with infected donors:			
Associated subgroup % infected (p-value)	Female 44% (0.02) Elderly male 50% (0.07)	Ate at restaurant B 31% (0.007)	Elderly (≥65)[b] 38% (0.02) Drinks much water 47% (0.004) Lives alone[b] 50% (0.001) At home during day[b] 39% (0.04) Seldom in large groups[b] 32% (0.007) Gastrointestinal condition history[b] 43% (0.003) Heart condition history[b] 36% (0.01)

[a] No infection detected during period of observation; fecal specimens were observed in at least half (i.e., six) of the specimen collection periods.

[b] Confounding among age, household size, occupation, group contact, heart conditions, and gastrointestinal conditions; only one of these factors may actually be related to donors infected with bacteria prominent in wastewater.

growth, and with negative to light growth of all bacteria recovered except E. coli (i.e., ''normal''). These data are presented in Table 73. Heavy Klebsiella levels in feces may be associated with an increased risk of gastrointestinal illness during the 2-week period of fecal donation and in the subsequent 4 weeks. However, since the illness rates for the heavy Klebsiella level are variable due to the small number of person-days observed, this observation of a risk ratio of about 3 for subsequent gastrointestinal illness in persons with a Klebsiella infection should be cautiously interpreted.

Episodes of Klebsiella infection coincided with two of the major wastewater irrigation periods: summer 1982 and summer 1983. Table 74 characterizes these infection episodes and presents the infection rates by aerosol exposure level. The statistical analysis of these infection episodes, denoted CKLB2X and CKLB2W for summer 1982 and CKLB4X and CKLB4W for summer 1983, for association with wastewater exposure is presented later.

Infections by Non-Klebsiella Category 2 Bacteria (Other Opportunistic Bacteria)

Infections by a variety of other possible opportunistic microbial pathogens also were detected: Staphylococcus aureus (4), Citrobacter freundii (3), Citrobacter diversus (2), and one each by API Group I, Candida albicans, Morganella morganii, Proteus mirabilis, Serratia fonticola, and Serratia liquefaciens. These infections occurred sporadically throughout the study (see Table 71). Donors who ate at restaurant B experienced significantly more of these infections (see Table 72). While not significantly associated ($p=0.11$) perhaps because of the small sample size, two (33%) rural donors drinking contaminated well water (see Section 5C for contamination criteria) had these opportunistic bacterial and fungal infections, while none of 11 rural donors drinking well water of better quality were infected.

An episode of infections by these opportunistic microorganisms occurred in the early spring of 1983 (see Table 74). While unrelated to any measure of wastewater exposure, it did appear to be associated with eating at least once per month at restaurant B ($p=0.009$).

Infections by Bacteria Prominent in Wastewater

The donor population experienced 27 infections by Aeromonas hydrophila and the fluorescent Pseudomonas species, some of the most prevalent enteric bacteria in the sprayed wastewater. Most (89%) of these infections were by the fluorescent Pseudomonas group (P. aeruginosa, P. fluorescens, and P. putida). As Table 71 shows, these infections occurred throughout 1982 but were more prevalent in 1983 when all of the A. hydrophila infections occurred.

The characteristics associated with the donors experiencing fluorescent Pseudomonas and A. hydrophila infections are presented in Table 72. The infected donors exhibited a pattern of characteristics associated with the elderly: age 65 and above, living alone, retirees and homemakers who spent the day at home, infrequent contact with large groups of people, previous gastrointestinal conditions, and previous heart conditions. Because many of these were characteristics of the same infected donors, the data

TABLE 73. ASSOCIATION OF LEVEL OF KLEBSIELLA GROWTH IN ROUTINE FECAL SPECIMENS[a] WITH THE INCIDENCE OF SELF-REPORTED ILLNESS IN THE PRIOR, CONCURRENT AND SUBSEQUENT BIWEEKLY REPORTING PERIODS

Level of Klebsiella growth	Period of illness observation	Person days observed	Incidence of self-reported illness (New illnesses/1000 person days) Rate (No. of new illnesses) Respiratory	Gastrointestinal	Skin
Heavy	DCP-1[c]	674	7.4 (5)	3.0 (2)	1.5 (1)
Moderate	DCP-1	1254	4.0 (5)	1.6 (2)	0 (0)
Neg to Light[b]	DCP-1	8460	5.2 (44)	2.2 (19)	0.6 (5)
Heavy	DCP[d]	679	4.4 (3)	4.4 (3)	0 (0)
Moderate	DCP	1318	7.6 (10)	2.3 (3)	0 (0)
Neg to Light	DCP	9997	6.5 (65)	1.5 (15)	0.2 (2)
Heavy	DCP+1[e]	674	1.5 (1)	5.9 (4)	1.5 (1)
Moderate	DCP+1	1335	7.5 (10)	1.5 (2)	0 (0)
Neg to Light	DCP+1	9429	4.9 (46)	2.2 (21)	1.2 (11)
Heavy	DCP+2[f]	672	4.5 (3)	7.4 (5)	1.5 (1)
Moderate	DCP+2	1335	3.0 (4)	2.2 (3)	0.7 (1)
Neg to Light	DCP+2	9530	6.3 (60)	2.4 (23)	0.2 (2)

[a] Includes routine fecal specimens donated from January 1982 (DCP 201) to August 1983 (DCP 317).

[b] Negative, very light or light for all bacteria except E. coli.

[c] Two-week illness observation period prior to donation of routine fecal specimen.

[d] Two-week illness observation period in which fecal specimen was donated.

[e] Two-week illness observation period after period of specimen donation.

[f] Two-week illness observation period after DCP+1.

TABLE 74. EPISODES OF BACTERIAL INFECTION DETECTED FROM ROUTINE FECAL SPECIMENS DURING IRRIGATION SEASONS

Period of observation	Irrigation period	Episode dependent variable name	Total donors observed	Number not infected[a]	Number (%) newly infected[b]	Infection rates, %, by aerosol exposure level		
						Low	Inter- mediate	High
KLEBSIELLA INFECTION EPISODES								
1982								
Jun 7-Sep 17	Jul 21-Sep 17	CKLB2W	88	75	13 (14.8)	13.6	20.4	0
(Aug 9-Sep 17)		CKLB2X	80	75	5^c (6.3)	5.0	9.3	0
1983								
Jun 6-Aug 18	Jun 29-Sep 20	CKLB4W	93	81	12 (12.9)	7.7	10.4	26.3
(Jul 18-Aug 18)		CKLB4X	89	81	8^c (9.0)	4.0	6.5	22.2
OTHER OPPORTUNISTIC BACTERIA INFECTION EPISODE								
1983								
Jan 31-Apr 22	Feb 15-Apr 30	COOB3	107	102	5 (4.7)	3.8	4.8	5.3
INFECTION EPISODES BY PROMINENT BACTERIA IN WASTEWATER								
1982								
Jan 4-Apr 2	Feb 16-Apr 30	CPBW1W	113	110	3 (2.7)	5.7	0	5.6
Jun 7-Sep 17	Jul 21-Sep 17	CPBW2W	89	85	4 (4.5)	0	4.1	11.1
(Aug 9-Sep 17)		CPBW2X	88	85	3^c (3.4)	0	4.1	5.9
1983								
Jun 6-Aug 18	Jun 29-Sep 20	CPBW4W	94	85	9 (9.6)	7.7	10.2	10.5

a Neither specimen from the individual during irrigation period contained the pathogen at a level classified as infected.

b Individuals infected during irrigation period, but not infected in previous month. Onset of the infection event was during the period of observation.

c Individuals whose infection event onset was definitely during irrigation period.

do not permit inference as to which one(s) may be actual susceptibility or exposure risk factors. Repeated or prolonged infections were observed in seven donors, six of whom were older than 60.

Drinking more water than others their age also appeared to be significantly associated with the infected donors. However, the quality of the drinking water of rural households with private wells was not associated with these infections in the subset of donors whose well water was monitored. Whereas two (20%) of the donors whose private wells were contaminated with the bacterial indicators experienced infections by these prominent wastewater bacteria, three (38%) of the donors who drank well water of better quality also had these infections.

The association the fluorescent Pseudomonas and A. hydrophila infections with self-reported illness is presented in Table 75. No patterns of association are evident, but only a small number of person-days of observation were available for donors with infections to these bacteria prominent in the wastewater. Footnotes h and i indicate that most (i.e., 6) of the illnesses in infected donors were reported by a single individual before and after one fluorescent Pseudomonas infection.

Episodes of infection by bacteria prominent in the wastewater occurred during three of the four wastewater irrigation periods monitored (see Table 74). The statistical analysis of these infection episodes is reported later.

H. CLINICAL VIROLOGY OF ROUTINE FECAL SPECIMENS

Viral isolates were recovered from routine fecal specimens by traditional tissue culture methods (see Figure 16). Enteroviruses were identified and typed by microneutralization procedures, while adenoviruses were identified by a group antigen-specific, fluorescent staining procedure. The prevalence and identification of viral isolates is presented in Table 76 by specimen collection period. The annual viral isolation rates are not directly comparable, both because of the addition of numerous adult donors in 1982 and 1983 to the predominantly child donor population of 1980 and 1981 and because of the different seasonal distribution of the specimens. The age-specific rates of viral recovery are presented in Table 77. Donors who were 0-5 years of age had substantially higher viral isolation rates than other age groups in each collection year. Older children (ages 6-17) also had higher virus recovery rates than adults. The viral isolation rate in the 0-5 age group was constant at 16-17% in 1981, 1982 and 1983. The higher isolation rates for children in 1980 (32% for ages 0-5 and 18% for ages 6-17) may be partially due to the restriction of the specimen collection to the summer months during 1980. Viral isolates were much less prevalent during 1983 than they had been in 1982 in all adult age groups and in school-age children.

The distribution of identified viral types differed by year, as Table 76 illustrates. Adenoviruses were the most prevalent type in 1982 and 1983, with the highest number of isolates recovered in January 1982. Coxsackie B and polioviruses were the most prevalent types in 1980, while polio-

TABLE 75. ASSOCIATION OF LEVEL OF GROWTH OF PROMINENT WASTEWATER BACTERIA[a] IN ROUTINE FECAL SPECIMENS[b] WITH THE INCIDENCE OF SELF-REPORTED ILLNESS IN THE PRIOR, CONCURRENT AND SUBSEQUENT BIWEEKLY REPORTING PERIODS

Level of growth of fl. Pseudomonas or A. hydrophila	Period of illness observation	Person days observed	Incidence of self-reported illness (New illnesses/1000 person days) Rate (No. of new illnesses)		
			Respiratory	Gastrointestinal	Skin
Heavy/Moderate	DCP-1[d]	216	4.6 (1)[h]	4.6 (1)[h]	0 (0)
Neg to Light[c]	DCP-1	8460	5.2 (44)	2.2 (19)	0.6 (5)
Heavy/Moderate	DCP[e]	225	0 (0)	4.4 (1)	0 (0)
Neg to Light	DCP	9997	6.5 (65)	1.5 (15)	0.2 (2)
Heavy/Moderate	DCP+1[f]	228	8.8 (2)[i]	13.2 (3)[i]	0 (0)
Neg to Light	DCP+1	9429	4.9 (46)	2.2 (21)	1.2 (11)
Heavy/Moderate	DCP+2[g]	214	0 (0)	0 (0)	0 (0)
Neg to Light	DCP+2	9530	6.3 (60)	2.4 (23)	0.2 (2)

a Fluorescent Pseudomonas and Aeromonas hydrophila.

b Includes routine fecal specimens donated from January 1982 (DCP 201) to August 1983 (DCP 317).

c Negative, very light or light for all bacteria except E. coli.

d Two-week illness observation period prior to donation of routine fecal specimen.

e Two-week illness observation period in which fecal specimen was donated.

f Two-week illness observation period after period of specimen donation.

g Two-week illness observation period after DCP+1.

h Both illnesses reported by ID 45201 in DCP 218.

i Both respiratory illnesses and two of the three gastrointestinal illnesses were reported by ID 45201 in DCP 220.

TABLE 76. PREVALENCE AND IDENTIFICATION OF VIRAL ISOLATES RECOVERED FROM ROUTINE FECAL SPECIMENS BY COLLECTION MONTH

Specimen collection period	Routine fecal donors	Viral isolation prevalence rate		Number of samples yielding designated viral type				
		Number	Percent	Adeno	Cox B	Echo	Polio	Unidentified
1980								
Jul	22	7	32	0	3	0	3	1
Aug	36	9	25	0	3	2	3	1
Sep	47	7	15	0	2	1	2	2
1980 Total	105	23	21.9	0	8	3	8	4
1981								
Apr/May[a]	27	0	0	0	0	0	0	0
Jun	45	5	11	2	0	0	3	0
Jul	30	6	20	2	0	1	1	2
Aug/Sep[a]	35	6	17	0	0	1	1	4
1981 Total	137	17	12.4	4	0	2	5	6
1982								
Jan 4-8	107	11	10.3	8	0	3	0	0
Mar 1-5	127	9	7.1	2	0	2	3	2
Mar 29-Apr 2	127	14	11.0	3	0	2	5	4
Jun 7-11	124	5	4.0	4	0	0	0	1
Aug 9-13	118	3	2.5	0	1	0	0	2
Sep 13-17	121	12	9.9	1	3	5	1	2
1982 Total	724	54	7.5	18	4	12	9	11
1983								
Jan 31-Feb 4	100	0	0	0	0	0	0	0
Apr 18-22	109	3	2.8	2	0	0	0	1
Jun 6-10	102	2	2.0	1	0	0	0	1
Jul 18-22	105	4	3.8	1	2	1	0	0
Aug 15-19	99	2	2.0	0	0	1	0	1
1983 Total	515	11	2.1	4	2	2	0	3

[a] Some donors provided more than one fecal specimen over this extended collection period. Tabulation based on first specimen donated.

TABLE 77. AGE-SPECIFIC ANNUAL RECOVERY OF VIRAL ISOLATES FROM ROUTINE FECAL SPECIMENS

Donor age, years	1980 Specimens	1980 Isolates	1980 (%)	1981 Specimens	1981 Isolates	1981 (%)	1982 Specimens	1982 Isolates	1982 (%)	1983 Specimens	1983 Isolates	1983 (%)
0-5	34	11	(32)	54	9	(17)	98	17	(17)	62	10	(16)
6-17	65	12	(18)	97	9	(9)	190	19	(10)	111	1	(1)
18-44	6	0	(0)	9	0	(0)	141	4	(3)	86	0	(0)
45-64							161	9	(6)	150	0	(0)
65+							134	5	(4)	106	0	(0)
All ages	105	23	(21.9)	160	18	(11.3)	724	54	(7.5)	515	11	(2.1)

and adenoviruses were most frequently recovered in 1981. Eight of the 22 poliovirus isolations were considered to be immunization-associated, in that the donor had received Sabin oral polio vaccine during the preceding month.

These patterns of viral recovery from healthy populations are consistent with other published studies conducted in the United States. In an early study reported by Honig and associates (1956), 92% of the enteric viruses isolated from healthy preschool children in Charleston, West Virginia were isolated over the period of June to October. In the lower socioeconomic group, 8.3% of specimens yielded viruses while only 3.1% of the samples from an upper middle class district were positive. Among the viruses isolated over a 29 month period, 44% were echoviruses; 37%, coxsackieviruses; and 19%, polioviruses.

Similarly, data collected by Gelfand and co-workers (1963), showed a seasonal pattern of enterovirus isolations among healthy children in six major U.S. cities over a two year period. In southern cities (Atlanta and Miami) enteroviruses were recovered year-round, albeit at lower frequencies in the winter season. In northern cities (Minneapolis, Buffalo and Seattle), virtually no viral isolations were made during late winter and early spring months. Positive viral isolation rates, excluding vaccine-derived polioviruses, ranged from 1% to as high as 22% among lower socioeconomic status children. Rates of viral isolation from males (12.6%) statistically exceeded that of females (9.5%) over the two year study. Excluding immunization-associated polioviruses, echoviruses accounted for 46% of the viral isolates; coxsackieviruses, 33%; polioviruses, 9%; and untypable isolates, 12%. Notably, the procedures used in this study to cultivate viral agents were not optimal for adenovirus recovery.

The occurrence of viruses within family units was described as part of the extensive Seattle Virus Watch program. Isolation rates of coxsackie-, echo- and adeno-viruses from fecal specimens provided by children 0-5 years of age averaged 5.3% as compared to 1.4% for children 6-9 years of age and 1% for mothers (Cooney et al., 1972). During this monitoring program a preponderance of isolates were vaccine-derived polioviruses. Of the nonpolioviruses recovered, adenoviruses accounted for appoximately 64% of the total fecal isolates while coxsackieviruses and echoviruses accounted for 20% and 16%, respectively.

The viral isolation results of all routine fecal specimens donated during each year of the LISS are presented in Tables 78 through 80 by participant for all individuals from whom a viral isolate was recovered. In some instances, the same viral type was shed and recovered in consecutive specimens collected approximately 4 weeks apart.

The association of viral infection, as determined by viral recovery from a routine fecal specimen, with self-reported illness was also investigated. The incidence densities of self-reported respiratory, gastrointestinal, and skin illnesses in the 2-week periods prior, concurrent, and subsequent to the fecal collection were compared for all routine fecal specimens with a viral isolate and with no viral isolate. This analysis was accumulated

TABLE 78. VIRAL ISOLATES RECOVERED FROM DONORS[a] OF ROUTINE FECAL SPECIMENS DURING BASELINE MONITORING
(July 1980 to September 1981)

ID	Viral isolates from fecal specimens									
number[a]	Period 015	Period 017	Period 019	Period 108	Period 110	Period 112	Period 114	Period 117	Period 118	Period 119
21111	-	Coxsackie B-3					unidentified			
43414	unidentified			-		-				
22712		-		-		-	unidentified	polio 1	-	
42711		polio 1	-			-				
30612	-	-	polio 1							
40812			unidentified	-		-				
53813		unidentified*		-		-			echo 11	
53911		echo 11*				-			unidentified	-
32412			-	-		adeno	-	-	unidentified	
56211	polio 3b	polio 1b		-			adeno		-	-
20211		-	polio 1			-			-	
21816		Coxsackie B-5	Coxsackie B-5		-					
21815		-	Coxsackie B-5			-				
45314			-	-	-	polio 3b	-	-	-	
45313							adeno			
10414	Coxsackie B-2		-	-		-	-	-	-	
55715			-	-		-	echo 5		unidentified	
55714				-		-	polio 3		-	
40411	Coxsackie B-3	Coxsackie B-3			-	-	-			
32112	Coxsackie B-3	-	-	-		-	-			
32111		-	-	-		polio 3b	-			
21012		-	unidentified							
21011	polio 3	-	-							
53313				-	-	-	-		unidentified	
43511		echo 24	echo 24							
40216		-	-			adeno			unidentified	
45112						polio 1b				
12211	polio 3	-	-	-	-	-		-	-	
43614		polio 1	-			-	-		-	

- No viral isolate recovered from fecal specimen
* Illness convalescent specimen
(Blank) No specimen obtained

[a] Only donors with viral isolates are listed.
b Recipient of oral vaccine during preceding month.

TABLE 79. VIRAL ISOLATES RECOVERED FROM DONORS[a] OF ROUTINE FECAL SPECIMENS IN 1982

ID number[a]	Fecal collection period in 1982 201 (Jan 4-8)	205 (Mar 1-5)	207 (Mar 29-Apr 2)	212 (Jun 7-11)	216 (Aug 9-13)	219 (Sep 13-17)
10201	adeno	–	polio 1	–	–	–
10414	adeno	–	+	–	+	–
10901	–	–	–	–	–	echo 27
11402	–	polio 3	–	–	–	–
11902	adeno	–	polio 1	–	–	echo 31
12211	–	–	–	–	–	echo 30
12501	adeno	–	–	–	–	–
12602	echo 5	–	–	–	–	–
13211	–	–	adeno	–		
13212		polio 3[b]	polio 3	–		
20502	adeno					
20713		–	–	–		adeno
21012				adeno	–	–
21112	adeno	adeno	echo 27		–	–
21301	–	–	–	–	–	CB 5
21611	echo 11	–	–	–	–	–
21915	–	adeno	–	–	–	–
21916	adeno	–	–	adeno	–	–
22712	adeno	echo 24	–	–	–	–
23112		+	–	–	–	–
23614		–	–	adeno	–	+
23615			adeno	–	–	–
32202	–	–	echo 17	–	–	
32411	–	–	–	adeno	–	–
32412	–	polio 1	adeno	–	–	–
40312	–	–	–	–	CB 4	CB 5
41302	–	–	–	–	–	polio 2
41601	echo 5	–	–	–	–	–
42801	–	–	polio 1	–	–	–
45113		–	+	–		
45312	–	–	–	+	–	+
45313	–	+	–	–	–	–
45314	–	–	–	–	+	CB 5
50501	–	echo 17	–	–	–	–
53901	–	–	+	–		–
53911	–	–	+	–		–
53912	–	–	–	–	–	echo 30
54502	–	–	–	–	–	echo 31
60111		–	polio 3[b]	–		–

(Blank) No fecal specimen obtained
– No viral isolate recovered from fecal specimen
+ Unidentified viral isolate recovered from fecal specimen

a Only donors with viral isolates are listed
b Recipient of oral vaccine during preceding month

TABLE 80. VIRAL ISOLATES RECOVERED FROM DONORS[a] OF ROUTINE FECAL SPECIMENS IN 1983

	Fecal collection period in 1983				
ID	303 (Jan 31-Feb 4)	308 (Apr 18-22)	312 (Jun 6-10)	315 (Jul 18-22)	317 (Aug 15-19)
20713	-	adeno	-	-	-
20714	-	adeno	-	-	-
21112	-	-	-	-	echo 27
32413	-	-	+	-	-
40216	-	-	-	CB 5	
45411		-	-	echo 15	
45412		+	-	CB 1	
60111	-		adeno	adeno	+

(Blank) No fecal specimen obtained
- No viral isolate recovered from fecal specimen
+ Unidentified viral isolate recovered from fecal specimen

[a] Only donors with viral isolates are listed.

over all routine fecal specimens provided in 1982 and 1983, when the donors represented all age groups and the illness data were more reliable. The results presented in Table 81 show that viral recovery from feces may be associated with an increased risk of respiratory illness during the 2-week period of fecal donation and during the subsequent 2-week period. Although the illness rates for positive viral isolates are variable due to the small number of person-days observed, this observation of a risk ratio of about 2 for concurrent and subsequent respiratory illness in persons with a viral isolate is consistent with the literature (Fox et al., 1977).

A viral infection event was defined as the isolation of a specific virus by laboratory cultivation in the second and not the first of consecutive routine fecal specimens from the same person. Subsequent recovery of the same virus in a specimen from the same individual was considered to be a new event if more than 6 weeks elapsed between sequential recoveries. Detection of a virus in the first of serial specimens was also considered a viral infection event.

Adenoviruses are often shed sporadically over an extended period of time. Thus, the time of onset of an adenovirus infection cannot be determined reliably from an adenovirus recovery in a specimen series. A poliovirus isolate recovered from a donor who received Sabin oral polio vaccine during the prior month was presumed to result from the immunization. Thus, the infection events to viruses other than adenoviruses or immunization-associated polioviruses whose onset was during periods of wastewater irrigation were identified to investigate their possible association with the donor's wastewater exposure.

Five episodes of infection by viruses other than adenoviruses and immunization-associated polioviruses that occurred during seasons of irrigation

TABLE 81. ASSOCIATION OF VIRAL ISOLATES IN ROUTINE FECAL SPECIMENS[a] WITH THE INCIDENCE OF SELF-REPORTED ILLNESS IN THE PRIOR, CONCURRENT AND SUBSEQUENT BIWEEKLY REPORTING PERIODS

Viral isolation	Period of illness observation	Person days observed	Incidence of self-reported illness (New illnesses/1000 person days) Rate (No. of new illnesses) Respiratory	Gastrointestinal	Skin
Positive	DCP-1[b]	409	4.9 (2)	2.4 (1)	0 (0)
Negative	DCP-1	8429	5.1 (43)	2.1 (18)	0.6 (5)
Positive	DCP[c]	600	11.7 (7)	3.3 (2)	0 (0)
Negative	DCP	9558	6.2 (59)	1.4 (13)	0.2 (2)
Positive	DCP+1[d]	588	10.2 (6)	3.4 (2)	0 (0)
Negative	DCP+1	9342	4.6 (43)	2.1 (20)	1.2 (11)
Positive	DCP+2[e]	581	5.2 (3)	0 (0)	1.7 (1)
Negative	DCP+2	9405	6.2 (58)	2.4 (23)	0.1 (1)

[a] Includes routine fecal specimens donated from January 1982 (DCP 201) to August 1983 (DCP 317).

[b] Two-week illness observation period prior to donation of routine fecal specimen.

[c] Two-week illness observation period in which fecal specimen was donated.

[d] Two-week illness observation period after period of specimen donation.

[e] Two-week illness observation period after DCP+1.

were detected from the routine fecal specimen virology. These viral infection episodes are described in Table 82. Three viral infection episodes occurred during periods of irrigation. Fifteen of the 120 donors monitored throughout the spring 1982 irrigation had at least one new viral infection. The onset of the viral infection definitely occurred after irrigation commenced for at least 9 of these 15 infected individuals (i.e., those nine in Table 79 in which the period 205 specimen was negative but a virus other than adeno or an immunization-associated polio was recovered from the period 207 specimen). The dependent variables for this episode were named CVIR1W for the observation period in which 15 individuals were infected and CVIR1X for the shorter observation period during irrigation in which 9 individuals were infected. Twelve of the 106 donors monitored during the summer 1982 irrigation period had at least one viral infection event (episode CVIR2). A viral infection episode (CVIR4W) also occurred in summer 1983. Viral infection episodes CVIR8 and CVIR9 occurring during summer 1980 and summer 1981 were also evaluated as nonirrigation control situations.

Infection rates are also presented by level of aerosol exposure in Table 82. Observed donors with a high level of aerosol exposure (AEI>5) during the summer 1982 irrigation exhibited a higher rate of viral infections (23.5%) than did donors with less aerosol exposure. The viral infection episodes occurring at other times did not show this pattern. The statistical analysis of these infection episodes for possible association of viral infections with wastewater irrigation is presented later.

TABLE 82. EPISODES OF INFECTION TO VIRUSES (EXCLUDING ADENOVIRUSES AND IMMUNIZATION-ASSOCIATED POLIOVIRUSES[a]) DETECTED FROM ROUTINE FECAL SPECIMENS DURING IRRIGATION SEASONS

Period of observation	Irrigation period	Episode dependent variable name	Total donors observed	Number not infected[b]	Number (%) newly infected[c]	Infection rates, %, by aerosol exposure level: Low	Intermediate	High
1980								
Jul 20-Sep 17	(None)	CVIR8	28	16	12 (42.9)	43	54	(0)
1981								
Jun 1-Sep 2	(None)	CVIR9	29	20	9 (31.0)	60	18	(0)
1982								
Jan 4-Apr 2	Feb 16-Apr 30	CVIR1W	120	105	15 (12.5)	10.5	13.8	11.8
(Mar 1-Apr 2)		CVIR1X	114	105	9[d] (7.9)	8.1	8.2	6.3
Jun 7-Sep 17	Jul 21-Sep 17	CVIR2W	106	94	12 (11.3)	7.7	9.5	23.5
(Aug 9-Sep17)		CVIR2X	105	94	11[d] (10.5)	7.7	8.1	23.5
1983								
Jun 6-Aug 18	Jun 29-Sep 20	CVIR4W	97	92	5 (5.2)	0	7.8	5.3

[a] Recipient of Sabin oral polio vaccine during prior month.

[b] Both of the individual's specimens during irrigation period were negative for viruses (other than adenoviruses).

[c] Individual had at least one viral infection event to a virus other than an adenovirus or immunization-associated poliovirus; onset was during the period of observation.

[d] Individuals whose infection event onset was definitely during irrigation period.

I. SEROLOGIC DATA AND SEROCONVERSION RATES

Antibody Prevalence

The frequency distribution of titers to all serologic agents which were used in this study is summarized in Table P-45 in Appendix P. It should be noted that the serologic testing protocol required assay of all of the bloods from only certain collection periods. Table 9 should be consulted to determine which bloods were included in the testing for each agent. With the exception of Norwalk and rotavirus, frequency distributions with small sample sizes (N<50) usually contain titers from individuals who provided blood samples on an irregular basis. Periods with small sample sizes also include titers which were obtained during retesting. Retesting was performed to confirm fourfold increases in titer and, whenever possible, to determine the exact interval of time when seroconversions occurred. It should be noted that 15% of the participants who provided paired bloods during the baseline period dropped out before the end of the study. Therefore, changes in the distribution of antibody titers between blood collection periods (for each of the agents) reflect the slight changes in the population as well as changes in antibody titer that resulted from infections in individuals that remained in the study.

Based on the first blood obtained from each study participant, approximately 85% of the entire study population had influenza A antibody. More than half of the study population had antibody to coxsackie B2, coxsackie B4, Legionella, and reovirus 2. Eighty-nine percent of the selected subpopulation (consisting of children under the age of 10 and high exposure adults with diarrhea in 1982) had antibody to rotavirus. Forty-two percent of the population had antibody to hepatitis A. The majority of the participants with hepatitis A antibody were over the age of 45 or resided in a lower socioeconomic status household. Seven of the 24 children (29%) and all 12 of the adults who were tested were found to have antibody to Norwalk virus. Less than 20% of the population had antibody to echoviruses 1, 17, 19, 20 and 24. Only about 1% of the population had antibody to E. histolytica. As would be expected, only a small portion of the population had no antibody to polioviruses 1 and 2. Forty percent of the participants had no detectable antibody to poliovirus 3.

Table P-45 in Appendix P should not be used to determine the efficiency of the poliovirus immunizations which occurred during the course of this study. The low poliovirus titers in blood samples that were collected in January and June 1982 were observed in infants, in adults who refused to be immunized, and in adults who had not provided a blood sample for testing earlier in the study. Table 83 illustrates the effect of immunization on the poliovirus titers of participants who were immunized (and provided paired bloods) during the baseline interval. The table also illustrates that the Salk vaccine series (without the booster) was more effective than the Sabin booster in increasing the level of antibody titer during the baseline period. Using Cochran-Mantel-Haenszel (nonzero correlation) statistics, the difference in vaccine effectiveness was found to be significant for all three poliovirus types ($p=0.003$ for polio 1, $p=0.001$ for polio 2 and $p<0.001$ for polio 3) during the baseline period. Since irrigation

TABLE 83. EFFECT OF IMMUNIZATION ON PARTICIPANT POLIOVIRUS TITERS BY AGENT AND VACCINE TYPE (FOR PARTICIPANTS WHO PROVIDED BLOOD IN BOTH THE BASELINE PERIOD AND IN JANUARY 1982)

	Poliovirus 1	Poliovirus 2	Poliovirus 3
Salk Vaccine[a]			
# immunized	68	68	68
# twofold increases in titer	11 (16%)	11 (16%)	9 (13%)
# fourfold or greater increases in titer	50 (74%)	52 (76%)	57 (84%)
Sabin Vaccine[b]			
# immunized	39	38	37
# twofold increases in titer	12 (31%)	7 (18%)	11 (30%)
# fourfold or greater increases in titer	17 (44%)	19 (50%)	15 (41%)

[a] Adults who were recommended for immunization received the complete Salk series. The majority of the adults (47/68) had received the first three Salk injections before the January 1982 blood was collected. The third injection was administered in June 1981. The booster was administered immediately after the blood sample was collected in January 1982.

[b] Children who were recommended for immunization received only the Sabin booster dose in May 1981, because all had previously received their basic immunization series.

began soon after the Salk booster was administered, the titer increases observed in the participants who received the boosters may have been caused either by the Salk booster or by exposure to wastewater aerosols. An analysis of their relative importance is presented later in the statistical results.

The frequency distribution of antibody titer by age group is listed in Table P-46 in Appendix P. Inspection of this table reveals that antibody presence remains constant among age groups for adenovirus 5; coxsackievirus B2; echoviruses 9, 11 and 20; Legionella; the polioviruses and reovirus 1. Antibody presence definitely increases with age for echoviruses 1 and 19, hepatitis A, influenza and reovirus 2. Antibody occurrence appears to increase from young children to older age groups for rotavirus, but the small sample sizes of the adult age categories render this impression uncertain.

Incidence Densities for Serologic Agents

The incidence density of infections (defined as a fourfold or greater increase in titer in paired sera), the incidence density ratio (IDR) and its 95% and 90% test-based confidence invervals were calculated as discussed in Section 4J. An infection incidence density ratio was considered to be significant if its 95% confidence interval did not include 1.0, provided the expected number of infections in both exposure groups compared was

2.0 or larger. The IDR was considered possibly significant if its 90% confidence interval did not include 1.0 and at least 2 infection events were expected in both groups compared.

No incidence density calculations or any statistical analyses were performed on results for coxsackieviruses A9 and B3, Norwalk agent, E. histolytica or hepatitis A. Due to a high prevalence of antibody to coxsackieviruses A9 and B3, serology testing was discontinued and no analysis was performed on the partial serologic results. There were three fourfold increases in titer to Norwalk agent. Two increases were observed during the irrigation period: one fourfold increase occurred in a high exposure level participant; the other increase occurred in a low exposure level participant. Unfortunately, the small sample size prevented interpretation of this information, and no further analyses were performed for the Norwalk data. There were two fourfold increases in E. histolytica titer: one during the baseline period and one during the January 1982-June 1983 time interval. The only hepatitis A infection identified during the course of the study occurred in the baseline period between June and December 1980. Thus, neither E. histolytica nor hepatitis A was included in further analyses.

Results were modified somewhat before incidence densities could be calculated for the polioviruses, the reoviruses, and Legionella. Only those participants who were not immunized were included in incidence density calculations for the three polioviruses. Thirty-four fourfold titer increases to reovirus 1 and 17 fourfold increases in titer to reovirus 2 were detected in the summer of 1982. Unfortunately, none of these particular fourfold increases were tested in pairs. Consequently, the titers associated with the unconfirmed infections were coded as missing and not included in either incidence density calculations or in any other statistical analyses. Therefore, although it appears that there were no reovirus infections in the summer 1982 irrigation season, in fact all of the (possible) positive results have been excluded. To conserve January 1982 blood for virus testing, bloods which were selected for use in the Legionella serologic testing (see Table 9) created interpretation problems because the exact 6-month interval in which the infection occurred was not identified. Whether the Legionella infections that occurred between June 1981 and June 1982 were incurred before or after irrigation commenced has not been determined. However, there were not enough Legionella infections to detect a significant difference (between exposure groups or between exposure levels) even if the exact 6-month interval of each seroconversion were known.

Table 84 compares the infection incidence densities to individual agents which were observed in the three aerosol exposure levels during the baseline and irrigation periods. The high exposure level was found to have the highest incidence density of infection for adenovirus 7 and echovirus 5 during the baseline period. The high exposure level was found to have the highest incidence density of infection for eight (coxsackieviruses B2 and B4, echoviruses 3, 11, 19, 20 and 24, and rotavirus) out of the nineteen agents during the irrigation period. As indicated in the table, the incidence density ratio of the high to the intermediate exposure levels was found to be possibly significant for coxsackievirus B4, as indicated by the 90% confidence interval. However, the incidence density ratios for

TABLE 84. COMPARISON OF BASELINE AND IRRIGATION INCIDENCE DENSITY RATES[a] BY WASTEWATER AEROSOL EXPOSURE LEVEL AND AGENT (NUMBER OF INFECTION EVENT INDICATED IN PARENTHESES)

	Baseline[b]			Irrigation[c]		
	Low exp level (AEI<1)	Med exp level (1≤AEI≤5)	Hi exp level (AEI>5)	Low exp level (AEI<1)	Med exp level (1≤AEI≤5)	Hi exp level (AEI>5)
Adeno 3	2.07 (2)	11.40 (11)	0.00 (0)	0.57 (1)	1.91 (7)	0.00 (0)
Adeno 5	3.16 (3)	5.27 (5)	0.00 (0)	1.15 (2)	2.58 (9)	1.17 (1)
Adeno 7	0.84 (1)	2.51 (3)	3.38 (2)	0.00 (0)	0.00 (0)	0.00 (0)
Cox B2	7.14 (7)	5.10 (5)	3.32 (2)	0.00 (0)	4.51 (7)	5.80 (2)
Cox B4	5.07 (5)	11.15 (11)	0.00 (0)	7.93 (6)	5.63 (9)	13.91 (5)[d]
Cox B5	0.82 (1)	6.57 (8)	3.44 (2)	1.67 (3)	3.62 (13)	2.28 (2)
Echo 1	0.85 (1)	5.11 (6)	0.00 (0)	0.57 (1)	0.00 (0)	0.00 (0)
Echo 3	8.29 (7)	4.15 (4)	1.80 (1)	3.98 (7)	4.19 (15)	5.75 (5)
Echo 5	0.96 (1)	0.96 (1)	1.82 (1)	0.00 (0)	0.28 (1)	0.00 (0)
Echo 9	1.64 (2)	4.11 (5)	3.44 (2)	1.19 (2)	0.00 (0)	0.00 (0)
Echo 11	5.85 (7)	6.69 (8)	3.47 (2)	4.48 (8)	3.79 (14)	7.91 (7)
Echo 17	1.05 (1)	1.05 (1)	0.00 (0)	0.00 (0)	0.83 (3)	0.00 (0)
Echo 19	0.00 (0)	3.21 (3)	0.00 (0)	0.00 (0)	0.83 (3)	1.18 (1)
Echo 20	1.05 (1)	4.19 (4)	0.00 (0)	1.18 (2)	1.97 (7)	2.31 (2)
Echo 24	2.15 (2)	6.46 (5)	1.80 (1)	2.98 (5)	2.77 (10)	4.66 (4)
Reo 1	14.22 (17)	12.55 (15)	5.07 (3)	2.99 (3)	5.75 (13)	1.94 (1)
Reo 2	7.53 (9)	17.57 (21)	11.80 (7)	2.93 (3)	5.19 (12)	0.00 (0)
Influenza A	3.24 (3)	12.96 (12)	7.34 (4)	10.33 (10)	13.29 (27)	8.04 (4)
Rotavirus	0.00 (0)	151.24 (7)	23.50 (4)	8.75 (1)	10.89 (7)	23.91 (8)

a Infection incidence density is expressed as the number of new infections per hundred person-years of observation:

$$\text{Infection ID} = \frac{\text{No. Fourfold Increases in Time Interval}}{\text{No. Person-days Observed During Interval}} \times 36525$$

b Spring 1982 aerosol exposure values were used for the baseline period (June 1980 to January 1982).

c Since an individual could have different exposures during the irrigation period (January 1982 to October 1983), the infection rate was calculated by summing results from each of the four irrigation seasons. Aerosol exposure values for 1982 or 1983 were used when it was not possible to determine the exact irrigation season in which the infection had occurred.

d The 90% confidence interval for the high to intermediate incidence density ratio does not include the value 1.

rotavirus and the other six enteroviruses was not found to be significant. In contrast, the incidence of influenza A infection (our epidemiologic control) was lowest in the high exposure level during the irrigation period. The majority of the ''susceptible'' study participants (i.e., children, adults over the age of 60, lower socioeconomic status families) was located in the intermediate and low exposure levels. Thus, this finding of elevated incidence of infections during the irrigation period to viruses recovered from the wastewater was not expected.

Table 85 compares the individual agent incidence densities for the two aerosol exposure groups during the baseline and irrigation periods. Since the ''susceptible'' population was more evenly divided between the two exposure groups, it was expected that there would be an even distribution of infections between the two groups. Nine agents were found to have a higher infection density in the high exposure group during the baseline period. The risk of echovirus 9 infection was six times greater for the high exposure group than the low exposure group; this ratio was found to be significant. The elevated risk of adenovirus 7 infection in the high exposure group was possibly significant during the baseline period. Eight agents were found to have a higher rate of infection in the high exposure group during the irrigation period. Infection rates were noticeably higher during the irrigation period for coxsackievirus B2, echoviruses 11 and 19, and rotavirus. The risk of infection for the high exposure group was found to be five times as great for coxsackievirus B2, twice as great for echovirus 11 and rotavirus, and seven times as great for echovirus 19, during the irrigation period. The elevated risks of infection by coxsackievirus B2 and echovirus 11 in the high exposure group were significant.

The agent groupings which were used in the serologic data analysis were defined in Table 18. Agents were grouped in order to increase the number of infections observed, thereby increasing the chances of detecting an association between infection and wastewater exposure that was operative for all agents in the group. For purposes of calculating incidence densities, incidence density ratios, and the associated 90% and 95% confidence intervals, it was assumed that the infections caused by the members of an agent grouping were independent events. Therefore each person was at risk of infection by each agent in the agent grouping during each period of observation. Thus, the person-days for each agent (agent-person-days) were considered to be additive. For example, if a person was observed for 100 days and there were three agents in the agent grouping, then that person was considered to be at risk to infection by the members of the agent grouping for 300 agent-person-days.

The assumption of independence of the infection events to the agents in each group is probably valid. Consideration was given to the possible confounding effects of virus-host interaction. While mixed infections with more than one enterovirus have been frequently observed in warm climates and under poor hygienic conditions (Parks et al., 1967), such multiple infections were found infrequently among normal families in the United States (Cooney et al., 1972). On the other hand, as demonstrated during live poliovirus vaccine trials, multiplication of one virus can effectively interfere with the growth of a second enterovirus (Sabin et al., 1960).

TABLE 85. COMPARISON OF BASELINE AND IRRIGATION ENTEROVIRUS INFECTION INCIDENCE DENSITY RATES[a] BY WASTEWATER AEROSOL EXPOSURE GROUP AND AGENT (NUMBER OF INFECTION EVENTS INDICATED IN PARENTHESES)

	Baseline[b]		Irrigation[c]	
	Low exp group (AEI<3)	High exp group (AEI≥3)	Low exp group (AEI<3)	High exp group (AEI≥3)
Adeno 3	3.96 (10)	2.10 (3)	1.53 (7)	0.56 (1)
Adeno 5	2.04 (5)	2.17 (3)	2.48 (11)	0.59 (1)
Adeno 7	0.69 (2)	2.65 (4)[e]	0.00 (0)	0.00 (0)
Cox B2	3.90 (10)	2.76 (4)	1.58 (3)	7.72 (6)[d]
Cox B4	3.87 (10)	4.07 (6)	6.63 (13)	8.79 (7)
Cox B5	1.76 (5)	4.13 (6)	3.08 (14)	2.23 (4)
Echo 1	1.42 (4)	2.15 (3)	0.22 (1)	0.00 (0)
Echo 3	3.96 (9)	2.16 (3)	4.41 (20)	4.03 (7)
Echo 5	0.39 (1)	1.44 (2)	0.22 (1)	0.00 (0)
Echo 9	0.70 (2)	4.82 (7)[d]	0.44 (2)	0.00 (0)
Echo 11	3.48 (10)	4.78 (7)	3.45 (16)	7.21 (13)[d]
Echo 17	0.80 (2)	0.00 (0)	0.66 (3)	0.00 (0)
Echo 19	0.80 (2)	0.72 (1)	0.22 (1)	1.72 (3)
Echo 20	1.60 (4)	0.72 (1)	2.03 (9)	1.14 (2)
Echo 24	2.43 (5)	2.10 (3)	2.70 (12)	3.98 (7)
Reo 1	10.07 (29)	4.03 (6)	4.18 (11)	5.00 (6)
Reo 2	9.01 (26)	7.41 (11)	4.10 (11)	3.27 (4)
Influenza A	5.96 (14)	4.75 (5)	11.42 (29)	11.86 (12)
Rotavirus	18.24 (4)	20.85 (7)	10.11 (6)	20.07 (10)

a Infection incidence density is expressed as the number of new infections per hundred person-years of observation:

$$\text{Infection ID} = \frac{\text{No. Fourfold Increases in Time Interval}}{\text{No. Person-days Observed During Interval}} \times 36525$$

b Spring 1982 aerosol exposure values were used for the baseline period (June 1980 to January 1982).

c Since an individual could have different exposures during the irrigation period (January 1982 to October 1983), the infection rate was calculated by summing results from each of the four irrigation seasons. Aerosol exposure values for 1982 or 1983 were used when it was not possible to determine the exact irrigation season in which the infection had occurred.

d The 95% confidence interval for the high to low group incidence density ratio does not include the value 1.

e The 90% confidence interval for the high to low group incidence density ratio does not include the value 1.

It was considered unlikely however that simultaneous, multiple infections would occur within the confines of a normal study population exposed to a presumably low viral infectious dose via environmental (aerosol) pathways.

Table 86 compares incidence densities of the three exposure levels for infections caused by agent groupings during the baseline and irrigation periods. It can be seen in Table 86 that the high exposure level had the lowest infection density for all agent groupings during the baseline period. Of more interest is the fact that the high exposure level had the highest infection density during the irrigation period for two of the three independent agent groupings: coxsackie B viruses and the echoviruses. The wastewater viruses, which consisted of coxsackie B and echoviruses (see Table 99), also caused the highest infection incidence density in the high exposure level during the irrigation period. The high exposure level's density of infection by wastewater viruses was found to be twice as great as the density of the intermediate exposure level; this result was significant because the 95% confidence interval for the high to intermediate WWV incidence density ratio exceeded 1.0. Since the wastewater viruses are a large subset of the serum neutralization viruses, it was not surprising to find that the high exposure level also had the highest rate of infection for the SNV grouping. The rate of infection to the SNV group in the high exposure level was found to be greater than the rate of infection in the low exposure level. Using the 90% confidence interval, the ratio of the incidence densities of the high exposure level to the low exposure level is possibly significant for the serum neutralization viruses. Given the demographics of the the study population and the distribution of infections during the baseline period, the higher rates of infection during the irrigation period were expected in the low or intermediate exposure levels. The high incidence density of infection observed in the high exposure level participants by the viruses which were recovered from the irrigation wastewater indicates an apparent association between exposure to irrigation wastewater aerosols and infection.

Table 87 compares incidence densities for the two exposure groups for infections caused by the same agent groupings. The high exposure group was found to have a slightly higher density of infection by all agent groupings during the baseline period. Comparison of Table 87 to Table 86 discloses that the higher baseline density of infections occurred among participants in the upper portion of the intermediate exposure level ($3<AEI\leq5$) rather than in the high exposure level. The high exposure group had the highest infection density for most of the same agent-groupings during the irrigation period. The incidence density pattern of infection to the wastewater-associated viruses during the irrigation period in the exposure groups is consistent with the exposure gradient seen in the exposure levels.

Table 88 lists the incidence densities for grouped agents for the three exposure levels during all time intervals of interest. Incidence densities for single agents are listed in Table P-47 in Appendix P. Close examination of the incidence densities reveals some distinct patterns. The high exposure participants experienced a higher density of infections by wastewater viruses, serum neutralization viruses, coxsackie B viruses, and echoviruses, particularly during the summer of 1982 and the entire

TABLE 86. COMPARISON OF BASELINE AND IRRIGATION INCIDENCE DENSITY RATES[a] BY WASTEWATER AEROSOL EXPOSURE LEVEL AND AGENT GROUPING

(Number of infection events indicated in parentheses)
[Number of infected individuals indicated in brackets]

	Baseline[b]			Irrigation[c]		
	Low exp level ($AEI<1$)	Med exp level ($1\leq AEI\leq 5$)	Hi exp level ($AEI>5$)	Low exp level ($AEI<1$)	Med exp level ($1\leq AEI\leq 5$)	Hi exp level ($AEI>5$)
SNV	2.62 (41)[31]	2.28 (82)[57]	1.53 (13)[10]	1.55 (37)[34]	1.94 (97)[84]	2.42 (29)[23][d]
WWV	2.99 (25)[23]	2.65 (51)[37]	1.32 (6)[5]	5.46 (24)[22]	4.68 (44)[42]	8.34 (17)[15][e]
POR				0.45 (4)[4]	0.79 (15)[15]	0.74 (3)[3]
ADEN	1.61 (5)[5]	2.63 (19)[17]	1.19 (2)[2]	0.57 (3)[3]	1.39 (15)[14]	0.38 (1)[1]
COXB	4.08 (13)[12]	3.31 (24)[22]	2.24 (4)[3]	2.76 (9)[9]	4.31 (29)[28]	5.73 (9)[8]
ECHO	2.46 (23)[19]	1.82 (39)[30]	1.39 (7)[5]	1.62 (25)[24]	1.63 (53)[45]	2.44 (19)[16]

a Infection incidence density is expressed as the number of new infections per hundred person-years of observation:

$$\text{Infection ID} = \frac{\text{No. Fourfold Increases in Time Interval}}{\text{No. Agent-Person-days Observed During Interval}} \times 36525$$

b Spring 1982 aerosol exposure values were used for the baseline period (June 1980 to January 1982).

c Since an individual could have different exposures during the irrigation period (January 1982 to October 1983), the infection rate was calculated by summing results from each of the four irrigation seasons. Aerosol exposure values from 1982 or 1983 were used when it was not possible to determine the exact irrigation season in which the infection had occurred.

d The 90% confidence interval for the high to low level incidence density ratio does not include the value 1.

e The 95% confidence interval for the high to intermediate incidence density ratio does not include the value 1.

TABLE 87. COMPARISON OF BASELINE AND IRRIGATION INCIDENCE DENSITY RATES[a] BY WASTEWATER AEROSOL EXPOSURE GROUP AND AGENT GROUPING

(Number of infection events indicated in parentheses)
[Number of infected individuals indicated in brackets]

	Baseline[b]		Irrigation[c]	
	Low exp group (AEI<3)	High exp group (AEI≥3)	Low exp group (AEI<3)	High exp group (AEI≥3)
SNV	2.08 (82)[62]	2.52 (54)[36]	1.79 (112)[99]	2.09 (51)[42]
WWV	2.42 (51)[44]	2.72 (31)[21]	4.88 (55)[51]	6.23 (30)[28]
POR			0.73 (17)[17]	0.54 (5)[5]
ADEN	2.04 (16)[15]	2.32 (10)[9]	1.25 (17)[16]	0.38 (2)[2]
COXB	3.13 (25)[23]	3.66 (16)[14]	3.59 (30)[29]	5.07 (17)[16]
ECHO	1.74 (41)[34]	2.20 (28)[20]	1.60 (65)[59]	2.03 (32)[26]

a Infection incidence density is expressed as the number of new infections per hundred person-years of observation:

$$\text{Infection ID} = \frac{\text{No. Fourfold Increases in Time Interval}}{\text{No. Agent-Person-days Observed During Interval}} \times 36525$$

b Spring 1982 aerosol exposure values were used for the baseline period (June 1980 to January 1982).

c Since an individual could have different exposures during the irrigation period (January 1982 to October 1983), the infection rate was calculated by summing results from each of the four irrigation seasons. Aerosol exposure values for 1982 or 1983 were used when it was not possible to determine the exact irrigation season in which the infection had occurred.

TABLE 88. INCIDENCE DENSITY RATES OF INFECTION FOR WASTEWATER AEROSOL EXPOSURE LEVELS BY AGENT GROUPING AND TIME INTERVAL
(Number of infections indicated in parentheses)
[When different than number of infections, number of infected individuals indicated in brackets]

Agent group Interval	Low exp level (AEI<1)	Med exp level (1<AEI<5)	High exp level (AEI>5)
ENV			
0-Baseline	2.62 (41)[31]	2.28 (82)[57]	1.53 (13)[10]
1-Spring 1982	1.40 (6)[5]	1.15 (13)	0.86 (2)
2-Summer 1982	0.61 (4)	1.20 (14)[13]	2.75 (7)[5][a]
3-Spring 1983	1.04 (4)	0.72 (7)	0.41 (1)
4-Summer 1983	0.55 (5)	1.64 (28)[20]	1.54 (7)[4][b]
5-1982	1.98 (25)[22]	2.13 (51)[[45]	3.87 (19)[14][c]
6-1983	1.97 (13)[11]	2.81 (43)[27]	3.34 (13)[9][b]
7-Irrigation	1.55 (37)[34]	1.94 (97)[84]	2.42 (29)[23][b]
WWV			
0-Baseline	2.99 (25)[23]	2.65 (51)[37]	1.32 (6)[5]
1-Spring 1982	1.97 (4)	1.53 (8)	0.00 (0)
2-Summer 1982	1.36 (3)	2.05 (8)[7]	7.02 (6)[5][c]
3-Spring 1983			
4-Summer 1983			
5-1982	2.14 (18)[17]	2.19(35)[32]	5.47 (18)[13][c]
6-1983	1.92 (2)	3.36 (8)	1.76 (1)
7-Irrigation	5.46 (24)[22]	4.68 (44)[42]	8.34 (17)[15][d]
POR			
0-Baseline			
1-Spring 1982	0.81 (3)	0.82 (8)	0.98 (2)
2-Summer 1982	0.19 (1)	0.75 (7)	0.49 (1)
3-Spring 1983			
4-Summer 1983			
5-1982	0.20 (1)	0.42 (4)	0.00 (0)
6-1983	0.48 (2)	0.74 (7)	0.41 (1)
7-Irrigation	0.45 (4)	0.79 (15)	0.74 (3)
ADEN			
0-Baseline	1.61 (5)	2.63 (19)[17]	1.19 (2)
1-Spring 1982	1.14 (1)	0.88 (2)	0.00 (0)
2-Summer 1982	0.75 (1)	0.87 (2)	0.00 (0)
3-Spring 1983	0.00 (0)	1.34 (3)	0.00 (0)
4-Summer 1983	0.00 (0)	0.25 (1)	0.00 (0)
5-1982	1.18 (3)	2.52 (12)[11]	0.00 (0)
6-1983	0.00 (0)	1.17 (4)	1.06 (1)
7-Irrigation	0.57 (3)	1.39 (15)[14]	0.38 (1)

continued...

TABLE 88. (CONT'D)

Agent group Interval	Low exp level (AEI<1)	Med exp level (1<AEI<5)	High exp level (AEI>5)
COXB			
0-Baseline	4.08 (13)[12]	3.31 (24)[22]	2.24 (4)[3]
1-Spring 1982	1.18 (1)	2.68 (6)	2.08 (1)
2-Summer 1982	0.75 (1)	2.15 (5)[4]	7.75 (4)[3][c]
3-Spring 1983	0.00 (0)	1.34 (1)	0.00 (0)
4-Summer 1983	2.76 (2)	4.59 (6)	0.00 (0)
5-1982	3.11 (8)	3.92 (19)[18]	9.79 (10)[8][c]
6-1983	3.77 (2)	5.92 (7)	3.73 (1)
7-Irrigation	2.76 (9)	4.31 (29)[28]	5.73 (9)[8]
ECHO			
0-Baseline	2.46 (23)[19]	1.82 (39)[30]	1.39 (7)[5]
1-Spring 1982	1.56 (4)	0.74 (5)	0.72 (1)
2-Summer 1982	0.51 (2)	1.00 (7)	1.97 (3)
3-Spring 1983	1.51 (4)	0.44 (3)	0.59 (1)
4-Summer 1983	0.48 (3)	1.77 (21)[14]	2.23 (7)[4]
5-1982	1.85 (14)[13]	1.40 (20)[19]	3.08 (9)[8]
6-1983	2.42 (11)[9]	2.99 (32)[19]	4.10 (11)[8][b]
7-Irrigation	1.62 (25)[24]	1.63 (53)[45]	2.44 (19)[16]

a The 95% confidence interval for the high to low level incidence density ratio does not include the value 1.

b The 90% confidence interval for the high to low level incidence density ratio does not include the value 1.

c The 95% confidence intervals for both the high to low level and high to intermediate incidence density ratios do not include the value 1.

d The 95% confidence interval for the high to intermediate incidence density ratio does not include the value 1.

year of 1982 (see Table 88). The majority of the infections which were observed during this period of time were caused by echovirus 11, and coxsackieviruses B4 and B5. These same agents were isolated from the irrigation wastewater at that time. Inspection of Table P-47 in Appendix P reveals that the high exposure level participants' incidence densities of infection by echovirus 11 and coxsackievirus B4 were significantly higher for 1982.

The unimmunized high exposure level participants had a noticeably higher rate of infection to poliovirus 1 during spring 1982 as shown in Table P-47. Poliovirus 1 was also isolated from the irrigation wastewater during spring 1982 (see Table 25). Poliovirus infections can occur as a result of exposure to a young child who has been recently immunized with oral polio vaccine. There were two cases, one during the baseline and the other in the high exposure level during spring 1982, where the infected adult lived in the household with a recently immunized child. However, since 64 of the 69 oral polio immunizations (administered to the study participants) occurred between May 1981 and July 1981, it would be expected that the poliovirus infection rate in non-immunized participants would have been higher during the baseline period than during the spring 1982 interval. This was not the situation which was observed: the infection incidence densities in unimmunized adults were higher in spring 1982 (see Table P-47).

During 1983 the high exposure level participants experienced the highest incidence density of infection by the serum neutralization viruses and echoviruses. Using a 90% confidence interval, the risk of infection by the serum neutralization viruses was found to be slightly greater and possibly significant for high exposure level participants (compared to the low exposure level) during 1983. The majority of the 1983 infections were caused by echoviruses 3, 11, 20, and 24. The risk of infection by echoviruses 20 and 24 was found to be seven times greater for high exposure level participants than for low exposure participants during the summer of 1983. None of those viruses were isolated from the wastewater in 1983, but less effort was placed on wastewater viral isolation in 1983 than in prior years.

Identified Serologic Infection Episodes

A serologic infection episode was defined as the observation of a sufficient number of fourfold (or greater) increases in antibody titer to an agent (or group of agents) within a given interval of time. The minimum number of infection events required to constitute a serologic infection episode was determined to be:

3 for agents recovered from the sprayed wastewater,
5 for agents not recovered from the sprayed wastewater.

A list of the serologic infection episodes which were observed, defined, and submitted to statistical analysis is presented later in Tables 98 and 99. Some donors experienced more than one infection during an infection episode. This occurred when the period of observation spanned three or more blood collection periods (allowing detection of multiple infections to the same agent) or when the infection episode involved a group of agents (allowing infections to several agents in the group). The guidelines used to determine the value of the dependent variable for a participant for each of the infection episodes were presented in Section 4.G.

J. OTHER INFECTIONS: MYCOBACTERIA, PARASITES AND CORONAVIRUS-LIKE PARTICLES

Non-tuberculosis Mycobacterial (NTM) Infections from Tuberculin Skin Testing

Mycobacteria infections were inferred from serial Mantoux tuberculin testing of the study population. The distribution of initial induration diameters of all tested participants is presented in Table 89. An increase in induration diameter from less than 5 mm to 5 mm or more was considered evidence of a new mycobacteria infection occurring in the interim. An increase in induration diameter from less than 5 mm to between 5 and 9 mm inclusive was treated as presumptive evidence of a new non-tuberculosis mycobacteria (NTM) infection. Indurations smaller than 5 mm in diameter are usually of non-mycobacterial origin, often due to trauma (A. Holguin, personal communication).

TABLE 89. PREVALENCE OF MYCOBACTERIA RESPONSE FROM INITIAL MANTOUX TUBERCULIN SKIN TEST RESULT

Size of induration	Number (percent) of responses
0 mm	367 (92.0)
1-4 mm	1 (0.3)
5-9 mm	8 (2.0)
≥10 mm	19 (4.8)
Self-reported previous reactor	4 (1.0)
TOTAL SURVEYED	399

The incidence of mycobacteria infections is summarized in Table 90. The tuberculin testing detected nine new mycobacteria infections in the study population during the study period, five of which were presumably due to NTM. Seven of the nine new mycobacteria infections observed occurred in the first year of the study, including four of the five presumed NTM infections. The incidence of mycobacteria infections was higher in the baseline period than in the irrigation period, both for the NTM and for all mycobacteria infections. There were insufficient mycobacteria infections after irrigation commenced to warrant statistical analysis. Only one of the detected mycobacteria infections clearly occurred after irrigation commenced. In a second case it is uncertain whether the onset of infection followed irrigation; in a third case (see footnote a of Table 90) it is uncertain whether there was a new infection. All three of these cases were Wilson residents with intermediate aerosol exposure and no direct wastewater contact. In summary, no evidence of association between mycobacteria infections and wastewater sprinkler irrigation was found.

TABLE 90. INCIDENCE OF MYCOBACTERIA INFECTIONS FROM TUBERCULIN TESTING OF STUDY POPULATION

		All mycobacteria infections	Presumed non-tuberculosis mycobacteria (NTM) infections
Infection criterion			
Change in induration diameter		<5 mm —> ≥5 mm	<5 mm —> 5-9 mm
Number of new infections by tuberculin testing interval			
DCP	Months		
012-113	6-80/6-81	7	4
113-225	6-81/12-82	0	0
225-320	12-82/10-83	1[a]	1
012-225[b]	6-80/12-82	1[b]	0
TOTAL		9[a]	5
Infection rate (= no. new infections/100 person-years at risk)			
Baseline (012-113)		3.6	2.1
Irrigation (225-320)		0.7[a] (1.4[c])	0.7

[a] Excludes ID 40201 with an induration series 8 mm, 0 mm, 11 mm, where the rise from 0 mm to 11 mm occurred from December 1982 to October 1983.

[b] New infection occurred between June 1980 and December 1982 (no tuberculin test obtained in June 1981).

[c] Including ID 40201.

Parasite Infestation

Previous studies of parasitic infections in occupational groups exposed to wastewater have produced variable results (Clark et al., 1984; Knobloch et al., 1983). However, one study in France found higher carriage rates of Entamoeba histolytica and Giardia intestinalis in sewer workers as compared to controls (Doby et al., 1980).

Stool specimens were collected from 206 participants during June, July or August 1983 to detect acute parasitic infestation. One of two portions of each specimen was mixed with polyvinyl alcohol and the other with 5% formalin to preserve trophozoites and cysts, respectively, for microscopic evaluation. The reagents were prepared and procedures for the ova-parasite (O-P) analyses were performed by Dr. Charles Sweet, Texas Department of Health.

Concurrently, 567 sera samples from 189 participants (3 sera from each participant obtained during June 1980, January 1982 and June 1983) were sent to Dr. George Healy, CDC, Atlanta, Georgia, for analysis of E. histolytica antibody. An indirect hemagglutination test (IHA) was used to detect invasive amebic disease.

The primary purpose of the O-P analysis and serosurvey was to determine if there was an association between contact with irrigation wastewater and having acute infestation or invasive infection by E. histolytica. The prevalence of other pathogenic protozoa and helminths was also of interest.

The results of the O-P survey are presented in Table 91. Protozoa were found in the fecal specimens from 21 (10.2%) of the routine specimen donors, which was relatively high for a population survey in Texas (C. Sweet, personal communication). Giardia lamblia were isolated from 5 (2.4%) of the specimens, but Entamoeba histolytica was not found.

Some clustering of protozoa within families was observed. G. lamblia was recovered from three of four members of household 122. Entamoeba coli was isolated from all five tested members of household 219. Two of the positive June donors from household 122 were retested in August with identical results.

The Giardia-positive donors had a significantly higher average aerosol exposure ($p=0.03$) than the Giardia-negative donors (see Table 92). However, all three donors with AEI>1 from whom G. lamblia was recovered were members of the same household (i.e., 122). The drinking water well of household 122 was contaminated with indicator bacteria during the survey months (see Table 46). While the two Giardia-positive children in this household were reported to drink bottled water only, ingestion via water used for food preparation or other household activities is still plausible. Since fecal contamination of the water supply and hand-to-mouth transfer of cysts from the feces of an infected individual are the major known modes of transmission of giardiasis (Benenson, 1975), they appear more likely routes than wastewater exposure. Also, in these circumstances, the members of household 122 cannot be considered independent observations, as assumed in the t-test. Thus,

TABLE 91. OVA AND PARASITE SURVEY OF LISS POPULATION

	Data collection period			
	312 Jun 6-10	315 Jul 18-22	317 Aug 15-19	Total
Number of donors of tested fecal specimens	101	87	18[a]	206[a]
Positive results:				
Chilomastix mesnili		40211		1 (0.5%)
Endolimax nana	12202 53201	40211	12202[b]	3[a] (1.5%)
Entamoeba coli	11812 12202 21915 21916 23602 42901 45411	23614 45101 45312	12202[b] 21902 21913 21914	13[a] (6.3%)
Entamoeba hartmanni		40211		1 (0.5%)
Giardia lamblia	12211	12201 12212 40214 55501	12211[b]	5[a] (2.4%)
Iodamoeba butschlii		52002		1 (0.5%)
Parasite infestation prevalence donors (%) positive	9 (8.9%)	9 (10.3%)	3[a] (17%)	21[a] (10.2%)

a Excludes positive specimens from persons with previous positive specimen.
b Retest result.

TABLE 92. AEROSOL EXPOSURE COMPARISON OF GIARDIA-POSITIVE AND GIARDIA-NEGATIVE FECAL DONORS IN OVA AND PARASITE SURVEY

	Routine fecal donors in ova and parasite survey		
	Giardia lamblia recovered	Negative for G. lamblia	Apparent Association (p-value)
Number of donors	5[a]	201	
Mean AEI	20.6	6.6	
Geometric mean AEI	3.02	1.53	Yes[a] (0.03)[b]

[a] Three of the fecal donors were from high AEI household 122 whose drinking water well was contaminated during the survey months.

[b] One-sided t test of difference in means in two independent populations; ln (AEI) transformation used to equalize variances.

the O-P results for G. lamblia are less likely to be associated with wastewater irrigation than with contaminated household drinking water and/or hand-to-mouth transfer of cysts.

The prevalence of antibody to E. histolytica in the IHA serosurvey was only about 1% (see Table P-45 in Appendix P). Only two seroconversions in adult males were determined in 189 participants tested (1.1%), which was a rather low rate. One conversion (ID 45101) occurred between June 1980 and January 1982 before irrigation began and the other (ID 21901) occurred between January 1982 and June 1983 after irrigation had started. Participant 21901 did not report any direct contact with wastewater and had an intermediate level of aerosol exposure in all three irrigation periods between January 1982 and June 1983. Neither acute nor invasive E. histolytica infestations were of an unusual magnitude. Thus, there was no evidence that wastewater contact was a source of E. histolytica infection to the participant population tested.

Electron Microscopy (EM) of Routine Fecal Specimens

HERL-Cincinnati received 370 routine fecal specimens for electron microscopic (EM) examination. Fecal viruses were visualized by EM using a negative staining technique.

The routine fecal specimens examined by EM were selected in a nonrandom proportional manner at UTSA from among those provided during each fecal collection period. Hence, they cannot be considered a representative sample of all routine fecal specimens donated.

In marked contrast to the variety of virus-like particles detected in illness specimens (see Table 66), coronavirus-like particles (CVLP) were the only virus-like particles detected in routine fecal specimens. Coronaviruses are pleomorphic, enveloped, RNA viruses which possess a fringe of distinctive projections resembling a solar corona. In humans, coronaviruses have chiefly been associated with respiratory illness, although as in several animal species they may have a role in gastroenteritis. The CVLP detected by EM in the Lubbock stools were of a highly pleomorphic type (see Figure 28) and possessed thin, knobbed-type projections rather than the more classical bulbous or petal-shaped projections. CVLP of the type detected here have been observed by other investigators; however, their significance as agents of human illness has not been firmly established (Macnaughton and Davies, 1981; Sitbon, 1985).

The occurrence of the CVLP positives observed in the routine fecal specimens examined is presented in Table 93. The detection rate was 7% to 8% in 1980 and 1981, 12% to 18% in 1982, and 0% to 2% in 1983. The specimen selection problem complicates interpretation of these prevalence rates, because the CVLP-positive donors tended to be closely followed in 1982, whereas few of their specimens from 1983 were selected for EM examination (see Table 94). Nevertheless, the data on positive donors still suggest that the prevalence of CVLP-like infections may have increased somewhat in 1982 and decreased somewhat in 1983.

All EM results for donors with CVLP-like detections are presented in Table 94. The persistence of positive results in most individuals over extended time periods is noteworthy (see IDs 21915, 21916, 40214, 40215, 45302 and 45314 for example). The clustering of infected donors within certain households (i.e., 207, 219, 402 and 453) is also apparent.

The age-specific prevalence of the CVLP infections is presented in Table 95. The prevalence of CVLP infections was inversely related to the age of the specimen donor. The occurrence in all routine specimens examined ranged from 18% in donors aged 0-5, to 8% in ages 6-17 and to 3% in adults. Because certain donors provided a substantial number of the positive detections (Table 94), age-specific prevalence among donors is also presented in Table 95 and the same age-related pattern was observed. The percentage of examined donors with CVLP detected was 21% in 0-5 year olds, 11% for ages 6-17 and 3% for adults. These rates are similar to the age-specific EM-positive prevalence rates for illness specimens (see Table 65), despite differences in the types of particles detected.

Comparison by inspection of the donors infected with CVLP to the donors whose routine fecal specimens were negative by EM suggests other characteristics may be associated with the infected donors. The more strongly associated characteristics of CVLP infected donors were a low socioeconomic status lifestyle and residence in Wilson. Most infected donors were also hispanics.

The occurrence of CVLP infections was high throughout 1982 and highest in the summer of 1982 (see Table 93), which were the year and season in which the study population had the highest exposure to wastewater irrigation. Table 96 compares the average aerosol exposure index (AEI) of donors detected to be shedding CVLP in routine fecal specimens during an irrigation period to the average AEI of donors of EM-negative routine fecal specimens during the same period. The donors with CVLP infections had less aerosol exposure than the EM-negative donors during the spring 1982 irrigation. While CVLP infected donors had a somewhat higher mean AEI than the EM-negative

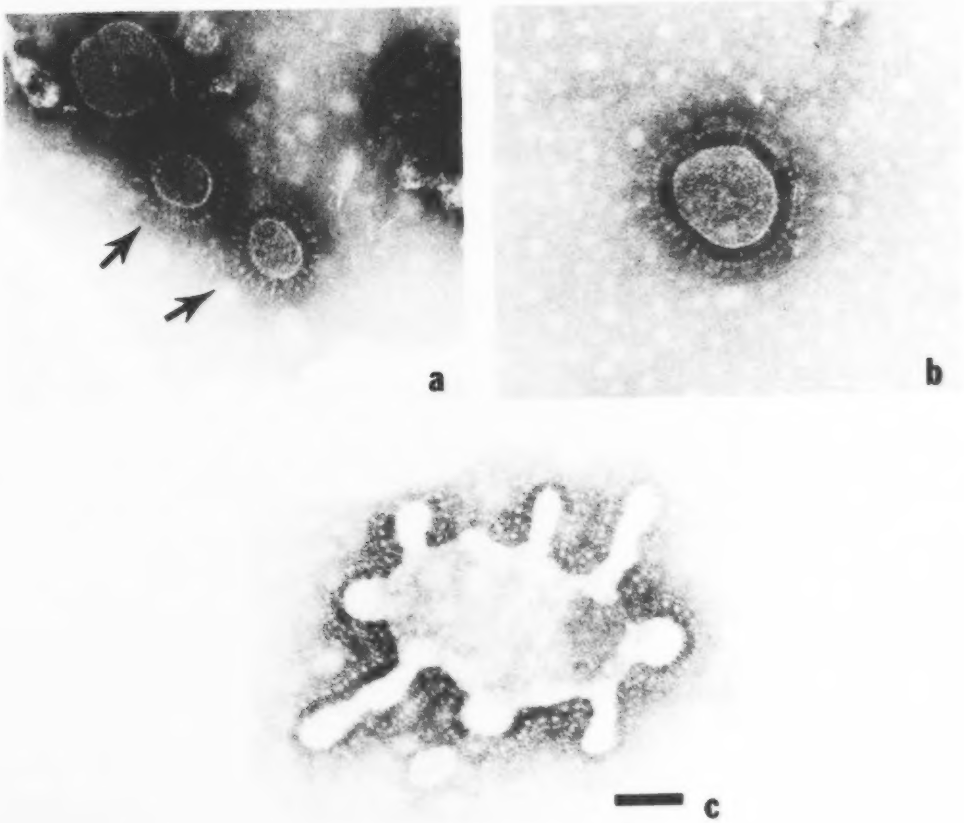

Figure 28. Coronavirus-like particles observed by EM in routine stool specimens. (a) Two particles (arrows) in the stool of 45314 (5-81). (b) A particle from the same individual collected over a year later (8-82). (c) A particle from 21916 showing the highly pleomorphic nature of the coronavirus-like particles detected in this study. Bar = 100 nm for a-c.

TABLE 93. OCCURRENCE OF CORONAVIRUS-LIKE PARTICLES IN ROUTINE FECAL SPECIMENS EXAMINED BY ELECTRON MICROSCOPY (EM)

Specimen collection quarter	Routine fecal specimens examined by EM	Infection prevalence rate		
		Coronavirus-like particles		Other[a] particles
		Number	Percent	
1980				
Jul-Sep	39	3	8	0
1981				
Apr-Jun	25	2	8	0
Jul-Sep	27	2	7	0
1982				
Jan-Mar	60	7	12	0
Apr-Jun	35	5	14	0
Jul-Sep	50	9	18	0
1983				
Jan-Mar	27	0	0	0
Apr-Jun	45	1	2	0
Jul-Sep	62	0	0	0
TOTAL	370	29	7.8	0

[a] Other characteristic virus-like particles which were observed by electron microscopy of illness stools include adeno-like, astro-like, calici-like, corona-like, Norwalk-like, and rota-like particles.

TABLE 94. ELECTRON MICROSCOPY RESULTS FOR ROUTINE FECAL SPECIMEN SERIES OF DONORS POSITIVE FOR CORONAVIRUS-LIKE PARTICLES

ID	Fecal collection period																				
number[a]	015	017	019	108	110	112	114	117	118	119	201	205	207	212	216	219	303	308	312	315	317
20713	+											0	0	0		0	0	0	–	0	–
20714	–										–	–	+	0		0	–	0	0	0	0
21514			+																		
21611											0	0	–	+	+	0					
21915		0	–			0					+	0	+	+	+	0	0	–	0	0	0
21916		+	0		0						+	0	0	+	+	+		0	0	0	0
30102											0	+	+	0	0						
40214						–			0			0	+	0	+						
40215		0	0			0			–			0	0		+		0	+	0	0	
43414	0			+		0															
45302														+	+	+					
45312			0	0	0	0	+	0	0		0	0	0	0	0	0					
45314			0	0	+	0	0	+	0		+	0	0	0	+	0	0	0	0	0	–

(Blank) No fecal specimen obtained
0 Fecal specimen obtained, but not analyzed by EM
– Negative by EM
+ Coronavirus-like particles detected by EM

[a] Only donors with virus-like particles detected by EM are listed.

TABLE 95. AGE-SPECIFIC PREVALENCE OF CORONAVIRUS-LIKE PARTICLES DETECTED BY ELECTRON MICROSCOPY IN ROUTINE FECAL SPECIMENS

	Occurrence in routine fecal specimens			Age-specific prevalence		
		Corona-like particles			Corona-like infected donors	
Donor age on 6-30-82, years	Examined by EM	No. positive	Percent	Donors Examined	Number	Percent
0-5	71	13	18	24	5	21
6-17	134	11	8	53	6	11
18-44	41	3	7	23	1	4
45-64	65	2	3	30	1	3
65+	59	0	0	21	0	0
All ages	370	29	7.7	151	13	8.6

TABLE 96. AVERAGE AEROSOL EXPOSURE COMPARISON OF CORONAVIRUS-LIKE INFECTED DONORS VERSUS NONINFECTED DONORS DURING IRRIGATION SEASONS IN 1982

		Mean AEI (No. of donors examined)		
Irrigation season	Routine fecal collection periods	Coronavirus-like infected donors[a]	Donors negative by EM[b]	Apparent association
Spring 1982	205, 207	2.28 (4)	5.33 (32)	No
Summer 1982	216, 219	3.47 (7)	2.25 (32)	No (p=.12)[c]

[a] Particles detected in one or both routine fecal specimens from observation period.

[b] All EM-examined routine specimens from donor in the period were negative.

[c] One-sided t test of difference in means in two independent populations; ln(AEI) transformation used to equalize variances.

donors during the summer 1982 irrigation, the difference was not statistically significant (see Table 96). Thus, the CVLP detections by EM provided no evidence of association with wastewater aerosol exposure.

K. OBSERVED EPISODES OF INFECTION

Infection Incidence Rates (IR) of Infection Episodes

The infection episodes detected by the LISS are presented in Tables 97-99. Procedures for defining infection events, infection status, and infection episodes were presented in Section 4.G. Each infection episode was uniquely specified by the method of detecting infections, the etiologic agent or agent group, and the period of observation relative to periods of irrigation. Acronyms of the specified components comprised the name of an infection episode's dependent variable (see Table 13). The value of the infection status dependent variable for each observed participant was the number of infection events detected in that individual during the observation period of the infection episode. A participant was seldom observed to experience more than one infection event to the agent (group) during the observation period of an infection episode, except in the serologic infection episodes to grouped agents over observation periods of 1 year or more (see the numbers of infection events and infected donors in Tables 97-99). To permit use of sensitive statistical methods requiring that the dependent variable only assume the values 0 or 1, all multiple infection events were treated as single infection events in most statistical analyses performed. Thus, a value of 0 indicated the donor was not infected during the period of observation while 1 indicated the donor was newly infected. The numbers of observed donors who were not infected and who were newly infected are provided in Tables 97-99 for each infection episode. These tables also present the infection incidence rates (IR) as percent infected for each infection episode. IR values varied widely during LISS observation periods, ranging from 1.0% for SE195 (echovirus 19 seroconversion rate for 1982) up to 42.9% for CVIR8 (clinical viral isolation rate for summer 1980). Most infection incidence rates were below 10%.

Infection episodes were classified as exposure situations when the observation period corresponded to one or two major irrigation periods and when the causative agent was found (or could be presumed) to be present in the wastewater at that time. The exposure infection episodes are listed in Table 100. Infection episodes were classified as control situations when the causative agent could not survive in wastewater (i.e., influenza A) or when the episode preceded the start of irrigation. The control infection episodes are given in Table 101. Each exposure and control infection episode listed in Tables 100 and 101 was statistically analyzed for association with wastewater aerosol exposure (see Section 5.L).

The infection incidence rates of both the low ($AEI<3$) and high ($AEI\geq3$) exposure groups and of all three exposure levels [low ($AEI<1$), intermediate ($1\leq AEI\leq5$) and high ($AEI>5$)]are also presented in Tables 100 and 101 for each infection episode. The risk ratio (RR) for exposure groups is the ratio of the infection rate in the high exposure group divided by the rate in the low exposure group. $RR=IR_{Hi}/IR_{Lo}$ values are presented in Tables

TABLE 97. CLINICAL INFECTION EPISODES

Clinical (C) agent group Irrigation period code	Period of observation	Recovered from sprayed wastewater?	Onset of infection events	Dependent variable name	Number of infection events	Fecal donors infected	Donors not infected	Infection incidence rate, %
Klebsiella, KLB								
2	Summer 1982[g]	Yes	X[a]	CKLB2X	5	5	75	6.3
			W[b]	CKLB2W	13	13	75	14.8
4	Summer 1983[i]	Yes[c]	X	CKLB4X	8	8	81	9.0
			W	CKLB4W	12	12	81	12.9
Other Opportunistic Bacteria, OOB								
3	Spring 1983[h]	No	X	COOB3	5	5	102	4.7
Prominent Bacteria in Wastewater, PBW								
1	Spring 1982[f]	Yes	W	CPBW1W	3	3	110	2.7
2	Summer 1982	Yes	W	CPBW2X	3	3	85	3.4
			X	CPBW2W	4	4	85	4.5
4	Summer 1983	Yes[c]	W	CPBW4W	9	9	85	9.6
All Viruses (excluding adeno and immunization polio), VIR								
	Sum 80 BL[d]	–	–	CVIR8	12	12	16	42.9
	Sum 81 BL[e]	–	–	CVIR9	11	9	20	31.0
1	Spring 1982	Some	X	CVIR1X	9	9	105	7.9
			W	CVIR1W	15	15	105	12.5
2	Summer 1982	Some	X	CVIR2X	11	11	94	10.5
			W	CVIR2W	14	12	94	11.3
4	Summer 1983	Some	W	CVIR4W	5	5	92	5.2
All Wastewater Isolates, WWI								
1	Spring 1982	Yes	X	CWWI1X	7	7	98	6.7
			W	CWWI1W	13	12	98	10.9
2	Summer 1982	Yes	X	CWWI2X	12	12	66	15.4
			W	CWWI2W	22	20	66	23.3
3	Spring 1983	Yes	X	CWWI3	4	4	100	3.8
4	Summer 1983	Yes[c]	W	CWWI4X	8	8	73	9.9
			W	CWWI4W	22	22	73	23.2

[a] X - onset of all infection events during irrigation period

[b] W - includes infection events whose onset may have preceded the irrigation period

[c] by inference from available wastewater data

[d] Sum 80 BL-Baseline: 7-21/9-17-80

[e] Sum 81 BL-Baseline: 6-1/9-2-81

[f] Spring 1982: 1-4/4-2-82

[g] Summer 1982: 6-7/9-17-82

[h] Spring 1983: 1-31/4-22-83

[i] Summer 1983: 6-6/8-19-83

TABLE 98. SEROLOGIC INFECTION EPISODES TO SINGLE AGENTS

Serologic (S) agent Irrigation period code	Period of observation	Recovered from sprayed wastewater?	Dependent variable name	Number of infection events	Blood donors infected	Donors not infected	Infection incidence rate, %
Adeno 3, AD3							
0	Baseline[a]	–	SAD30	13	13	242	5.1
5	1982[d]	–	SAD35	7	7	297	2.3
Adeno 5, AD5							
0	Baseline	–	SAD50	7	7	239	2.8
5	1982	–	SAD55	8	8	285	2.7
Adeno 7, AD7							
0	Baseline	–	SAD70	6	6	297	2.0
Coxsackie B2, CB2							
0	Baseline	–	SCB20	14	14	230	5.7
5	1982	Yes	SCB25	9	9	284	3.1
Coxsackie B4, CB4							
0	Baseline	–	SCB40	16	16	227	6.6
2	Summer 1982[c]	Yes	SCB42	5	5	284	1.7
5	1982	Yes	SCB45	20	19	281	6.3
Coxsackie B5, CB5							
0	Baseline	–	SCB50	11	11	276	3.8
1	Spring 1982[b]	Yes	SCB51	4	4	305	1.3
2	Summer 1982	Yes	SCB52	4	4	304	1.3
5	1982	Yes	SCB55	8	8	288	2.7
4	Summer 1983[f]	Yes	SCB54	8	8	248	3.1
6	1983[g]	Yes	SCB56	9	9	247	3.5
Echo 1, E01							
0	Baseline	–	SE010	7	7	285	2.4
Echo 3, E03							
0	Baseline	–	SE030	13	12	247	4.6
5	1982	No	SE035	9	9	288	3.0
4	Summer 1983	No	SE034	11	11	241	4.4
6	1983	No	SE036	18	18	239	7.0
Echo 9, E09							
0	Baseline	–	SE090	9	8	268	2.9
Echo 11, E11							
0	Baseline	–	SE110	17	17	271	5.9
1	Spring 1982	Yes	SE111	4	4	298	1.3
2	Summer 1982	Yes	SE112	7	7	296	2.3
5	1982	Yes	SE115	19	19	283	6.3
4	Summer 1983	No	SE114	6	6	249	2.4
6	1983	No	SE116	10	10	249	3.9

continued...

TABLE 98. (CONT'D)

Serologic (S) agent Irrigation period code	Period of observation	Recovered from sprayed wastewater?	Dependent variable name	Number of infection events	Blood donors infected	Donors not infected	Infection incidence rate, %
Echo 19, E19							
5	1982	Yes	SE195	3	3	291	1.0
Echo 20, E20							
0	Baseline	–	SE200	5	5	265	1.9
4	Summer 1983	No	SE204	6	6	241	2.4
6	1983	No	SE206	9	9	241	3.6
Echo 24, E24							
0	Baseline	–	SE240	9	8	261	3.0
5	1982	Yes	SE245	7	7	287	2.4
4	Summer 1983	No	SE244	7	7	244	2.8
6	1983	No	SE246	12	10	242	4.0
Polio 1, PL1							
0	Baseline	–	SPL10	70^{h}	70	175	28.6
		Adults Salk	immunized:	50	50	18	73.5
		Adults not	immunized:	2	2^{i}	97	2.0
		Children Sabin	immunized:	17	17	22	43.6
		Children not	immunized:	1	1^{i}	38	2.6
1	Spring 1982	Yes	SPL11	13^{h}	13	234	5.3
		Polio	immunized:	8	8	53	13.1
		Not	immunized:	5	5	181	2.7
Polio 2, PL2							
0	Baseline	–	SPL20	73^{h}	73	169	30.2
		Adults Salk	immunized:	52	52	16	76.4
		Adults not	immunized:	0	0^{i}	98	0
		Children Sabin	immunized:	19	19	19	50.0
		Children not	immunized:	2	2^{i}	36	5.3
1	Spring 1982	Yes	SPL21	9^{h}	9	235	3.7
		Polio	immunized:	7	7	54	11.5
		Not	immunized:	2	2^{i}	181	1.1

continued...

TABLE 98. (CONT'D)

Serologic (S) agent Irrigation period code	 Period of observation	Recovered from sprayed wastewater?	Dependent variable name	Number of infection events	Blood donors infected	Donors not infected	Infection incidence rate, %
Polio 3, PL3							
0	Baseline	–	SPL30	72[h]	72	169	29.9
		Adults Salk	immunized:	57	57	11	83.8
		Adults not	immunized:	0	0[i]	98	0
		Children Sabin	immunized:	15	15	22	40.5
		Children not	immunized:	0	0[i]	38	0
1	Spring 1982	Yes	SPL31	7[h]	7	236	2.9
		Polio	immunized:	7	7	54	11.5
		Not	immunized:	0	0[i]	182	0
Reovirus 1, RE1							
0	Baseline	–	SRE10	35	35	246	12.5
1	Spring 1982	–	SRE11	16	16	297	5.1
Reovirus 2, RE2							
0	Baseline	–	SRE20	37	37	241	13.3
1	Spring 1982	–	SRE21	13	13	297	4.2
Rotavirus, ROT							
0	Baseline	–	SROT0	13	11	19	36.7
1	Spring 1982	–	SROT1	3	3	45	6.3
2	Summer 1982	–	SROT2	4	4	50	7.4
5	1982	–	SROT5	7	7	45	13.5
3	Spring 1983[e]	–	SROT3	3	3	45	6.3
4	Summer 1983	–	SROT4	6	6	39	13.3
6	1983	–	SROT6	9	9	35	20.5
Legionella, LEG							
	6-81/6-83	No	SLEG7	6	6	207	2.8
Influenza A, INA							
0	6-80/6-81	–	SINA0	19	19	167	10.2
1	6-81/6-82	–	SINA1	6	6	229	2.6
3	6-82/6-83	–	SINA3	35	35	219	13.8

a Baseline: 6-80/1-82

b Spring 1982: Jan-Jun 1982

c Summer 1982: Jun-Dec 1982

d 1982: Jan-Dec 1982

e Spring 1983: Dec 1982-Oct 1983

f Summer 1983: Jun-Oct 1983

g 1983: Dec 1982-Oct 1983

h Includes polio immunization seroconversion

i Not an infection episode (too few infected donors)

TABLE 99. SEROLOGIC INFECTION EPISODES TO GROUPS OF AGENTS

Serologic (S) agent Group Irrigation period code	Period of observation	Specific agents included Adeno	 Cox B	 Echo	Dependent variable name	Number of infection events	Blood donors infected	Donors not infected[a]	Infection incidence rate, %
Sporadic Serum Neutralization Tested Viruses, POR									
0	Baseline			5,17,19	SPORO	8	8	207	3.7
1	Spring 1982	3,5,7	2,4	1,3,5,9,17, 19,20,24	SPOR1	13	13	175	6.9
2	Summer 1982	3,5,7	2	1,3,5,9,17, 19,20,24	SPOR2	9	9	199	4.3
5	1982	7		1,5,9,17,20	SPOR5	5	5	232	2.1
6	1983	3,5,7		1,5,9,17,19	SPOR6	10	10	218	4.4
All Viruses in Sprayed Wastewater, WWV									
1	Spring 1982		5	1,5,11,17, 19,20	SWWV1	12	12	210	5.4
2	Summer 1982		2,4,5	11,24	SWWV2	16	15	235	6.0
5	1982		2,4,5	1,5,11,17, 19,20,24	SWWV5	70	62	173	26.4
6	1983		5	19	SWWV6	11	11	235	4.5
All Serum Neutralization Tested Viruses, SNV									
0	Baseline	3,5,7	2,4,5	1,3,5,9,11, 17,19,20,24	SSNVO	136	98	110	47.1
1	Spring 1982	3,5,7	2,4,5	1,3,5,9,11, 17,19,20,24	SSNV1	21	20	163	10.9
2	Summer 1982	3,5,7	2,4,5	1,3,5,9,11, 17,19,20,24	SSNV2	24	22	168	11.6
5	1982	3,5,7	2,4,5	1,3,5,9,11, 17,19,20,24	SSNV5	94	81	144	36.0
3	Spring 1983	3,5,7	5	1,3,5,9,11, 17,19,20,24	SSNV3	12	12	200	5.7
4	Summer 1983	3,5,7	5	1,3,5,9,11, 17,19,20,24	SSNV4	40	29	180	13.9
6	1983	3,5,7	5	1,3,5,9,11, 17,19,20,24	SSNV6	69	47	174	21.3

[a] Donors without seroconversions excluded unless their seroconversion status to all specific agents listed was observed.

TABLE 100. INFECTION INCIDENCE RATES[a] BY EXPOSURE GROUPS AND LEVELS AND RISK RATIO SCORE OF INFECTION EPISODES CLASSIFIED AS EXPOSURE SITUATIONS

''Exposure'' infection episode			Jointly independent episode group[b]	Infection incidence[a]		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							Risk ratio score[c]
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable		No.	%	Low (<3) IR	High (>3) IR	AEI Group RR	Low (<1) IR	Inter-mediate IR	High (>5) IR	AEI Level RR	
Clinical (C)													
KLB (Klebsiella)													
	2 (Sum 82)	CKLB2X	A	5	6.3	5.1	9.5	1.9	5.0	9.3	0	0	0
		CKLB2W		13	14.8	13.8	17.4	1.3	13.6	20.4	0	0	0
	4 (Sum 83)	CKLB4X	A	8	9.0	4.6	20.8	4.5	4.0	6.5	22.2	5.6	++
		CKLB4W		12	12.9	7.5	26.9	3.6	7.7	10.4	26.3	3.4	++
OOB (Other opportunistic bacteria)													
	3 (Spr 83)	COOB3	A	5	4.7	3.3	6.4	1.9	3.8	4.8	5.3	1.4	0
PBW (Prominent bacteria in wastewater)													
	1 (Spr 82)	CPBW1W	A	3	2.7	2.8	2.4	0.8	5.7	0	5.6	1.0	0
	2 (Sum 82)	CPBW2X	A	3	3.4	3.1	4.3	1.4	0	4.1	5.9	Large	0
		CPBW2W		4	4.5	3.1	8.3	2.7	0	4.1	11.1	Large	+
	4 (Sum 83)	CPBW4W	A	9	9.6	8.8	11.5	1.3	7.7	10.2	10.5	1.4	0
VIR (Viruses, excluding adeno and immunization polio)													
	1 (Spr 82)	CVIR1X	A	9	7.9	8.3	7.1	0.9	8.1	8.2	6.3	0.8	0
		CVIR1W		15	12.5	14.3	9.3	0.7	10.5	13.8	11.8	1.1	0
	2 (Sum 82)	CVIR2X	A	11	10.5	7.6	19.2	2.5	7.7	8.1	23.5	3.1	++
		CVIR2W		12	11.3	8.8	19.2	2.2	7.7	9.5	23.5	3.1	+
	4 (Sum 83)	CVIR4W	A	5	5.2	5.6	4.0	0.7	0	7.8	5.3	Large	0
WWI (Agents isolated from wastewater)													
	1 (Spr 82)	CWWI1X	D	7	6.7	6.2	7.5	1.2	6.3	5.3	12.5	2.0	0
		CWWI1W		12	10.9	11.6	9.8	0.8	14.3	6.9	17.6	1.2	0
	2 (Sum 82)	CWWI2X	D	12	15.4	13.6	21.1	1.6	5.0	20.9	13.3	2.7	0
		CWWI2W		20	23.3	20.3	31.8	1.6	13.6	29.2	18.8	1.4	0

continued...

TABLE 100. (CONT'D)

''Exposure'' infection episode			Jointly independent episode group^b	Infection incidence^a		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							Risk ratio score^c
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable		No.	%	Low (<3) IR	High (≥3) IR	AEI Group RR	Low (<1) IR	Inter-mediate IR	High (>5) IR	AEI Level RR	
WWI (Cont'd)													
	3 (Spr 83)	CWWI3	D	4	3.8	3.4	4.4	1.3	3.8	3.3	5.6	1.4	0
	4 (Sum 83)	CWWI4X	D	8	9.9	5.0	23.8	4.8	4.3	7.1	25.0	5.8	++
		CWWI4W		22	23.2	17.4	38.5	2.2	15.3	22.0	36.8	2.4	+
Serologic (S)													
AD3 (Adeno 3)													
	5 (1982)	SAD35	B	7	2.3	2.7	1.4	0.5	1.1	3.5	0	0	0
AD5 (Adeno 5)													
	5 (1982)	SAD55	B	8	2.7	3.6	0	0	2.2	3.7	0	0	0
CB2 (Coxsackie B2)													
	5 (1982)	SCB25	B	9	3.1	1.8	6.9	3.7	0	4.2	5.6	Large	+
CB4 (Coxsackie B4)													
	2 (Sum 82)	SCB42	A	5	1.7	1.3	3.0	2.3	1.1	1.2	5.9	5.2	+
	5 (1982)	SCB45	B	18	6.1	5.4	8.1	1.5	8.0	4.1	11.1	1.4	0
CB5 (Coxsackie B5)													
	1 (Spr 82)	SCB51	A	4	1.3	1.0	1.8	1.8	1.3	1.6	0	0	0
	2 (Sum 82)	SCB52	A	4	1.3	0.8	2.8	3.3	0	1.2	5.1	Large	+
	5 (1982)	SCB55	B	8	2.7	2.3	4.2	1.8	1.1	2.5	8.1	7.5	0
	4 (Sum 83)	SCB54	A	8	3.1	4.1	0	0	2.6	4.2	0	0	0
	6 (1983)	SCB56	B	9	3.5	4.3	1.4	0.3	2.9	4.0	2.6	0.9	0
E03 (Echo 3)													
	5 (1982)	SE035	B	9	3.0	3.6	1.4	0.4	4.4	2.4	2.8	0.6	–
	4 (Sum 83)	SE034	A	11	4.4	4.1	5.2	1.3	1.4	5.7	5.4	4.0	0
	6 (1983)	SE036	B	18	7.0	5.9	9.9	1.7	4.3	7.4	10.3	2.4	+

continued...

TABLE 100. (CONT'D)

''Exposure'' infection episode			Jointly independent episode group[b]	Infection incidence[a]		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							Risk ratio score[c]
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable		No.	%	Low (<3) IR	High (≥3) IR	AEI Group RR	Low (<1) IR	Intermediate IR	High (>5) IR	AEI Level RR	
E11 (Echo 11)													
	1 (Spr 82)	SE111	A	4	1.3	1.0	1.9	1.9	2.5	1.1	0	0	0
	2 (Sum 82)	SE112	A	7	2.3	2.1	2.9	1.4	2.1	1.7	5.9	2.9	0
	5 (1982)	SE115	B	19	6.3	4.8	10.8	2.2	5.3	4.7	16.2	3.0	+
	4 (Sum 83)	SE114	A	6	2.4	1.5	5.1	3.3	1.4	2.8	2.6	1.9	0
	6 (1983)	SE116	B	10	3.9	2.7	6.9	2.6	4.3	3.3	5.0	1.2	+
E19 (Echo 19)													
	5 (1982)	SE195	B	3	1.0	0.5	2.7	6.0	0	1.2	2.9	Large	+
E20 (Echo 20)													
	4 (Sum 83)	SE204	A	6	2.4	2.1	3.6	1.7	0	2.9	5.3	1.8	0
	6 (1983)	SE206	B	9	3.6	3.9	2.8	0.7	3.0	3.5	5.1	1.7	0
E24 (Echo 24)													
	5 (1982)	SE245	B	7	2.4	2.7	1.4	0.5	4.7	1.2	2.8	0.6	-
	4 (Sum 83)	SE244	A	7	2.8	1.6	6.9	4.4	0	3.5	5.4	Large	+
	6 (1983)	SE246	B	10	4.0	2.2	8.6	3.9	1.5	4.0	7.9	5.1	+
PL1 (Polio 1)													
	1 (Spr 82)	SPL11		13	5.3	2.0	10.4	5.2	0	4.5	15.8	Large	++
	61 polio immunized:		A	8	13.1	3.6	21.2	5.9	0	14	20	Large	+
	186 not immunized:		A	5	2.7	1.6	4.8	2.9	0	1.7	13.0	Large	+
PL2 (Polio 2)													
	1 (Spr 82)	SPL21		9	3.7	1.3	7.4	5.7	3.8	2.0	10.5	2.8	++
	61 polio immunized:		A	7	11.5	3.6	18.2	5.1	10	8	20	2.0	+
PL3 (Polio 3)													
	1 (Spr 82)	SPL31		7	2.9	2.0	4.2	1.4	1.9	3.3	2.6	1.4	0
	61 polio immunized:		A	7	11.5	10.7	12.1	1.1	10	14	7	0.7	0

continued...

TABLE 100. (CONT'D)

''Exposure'' infection episode			Jointly independent episode group[b]	Infection incidence[a]		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							Risk ratio score[c]
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable		No.	%	Low (<3) IR	High (≥3) IR	AEI Group RR	Low (<1) IR	Intermediate IR	High (>5) IR	AEI Level RR	
RE1 (Reo 1)													
	1 (Spr 82)	SRE11	A	16	5.1	5.4	4.5	0.8	3.7	6.3	2.5	0.7	0
RE2 (Reo 2)													
	1 (Spr 82)	SRE21	A	13	4.2	5.0	2.7	0.5	3.8	5.3	0	0	0
ROT (Rotavirus)													
	1 (Spr 82)	SROT1	A	3	6.3	4.2	8.3	2.0	0	7	8	Large	0
	2 (Sum 82)	SROT2	A	4	7.4	2.8	16.7	6.0	10	0	21	2.1	+
	5 (1982)	SROT5	B	7	13.5	9.4	20.0	2.1	10	11	21	2.1	+
	3 (Spr 83)	SROT3	A	3	6.3	4.8	7.4	1.6	0	6	7	Large	0
	4 (Sum 83)	SROT4	A	6	13.3	12.5	14.3	1.1	0	12	19	Large	0
	6 (1983)	SROT6	B	9	20.5	20.0	20.8	1.0	25	17	25	1.0	0
LEG (Legionella pneumophila 1)													
	1981-83	SLEG7	B	6	2.8	2.7	3.1	1.1	0	4.1	3.0	0.7	0
POR (Sporadic serum neutralization viruses)													
	1 (Spr 82)	SPOR1	A	13	6.9	7.3	6.3	0.9	6.5	6.9	7.7	1.2	0
	2 (Sum 82)	SPOR2	A	9	4.3	4.9	2.3	0.5	1.5	6.0	3.8	2.5	0
	5 (1982)	SPOR5	B	5	2.1	2.7	0	0	1.4	3.0	0	0	0
	6 (1983)	SPOR6	B	10	4.4	4.9	3.0	0.6	3.3	5.3	2.8	0.8	0
WWV (Viruses isolated from wastewater)													
	1 (Spr 82)	SWWV1	D	12	5.4	5.5	5.3	1.0	6.8	6.0	0	0	0
	2 (Sum 82)	SWWV2	D	15	6.0	5.2	8.8	1.7	3.8	4.9	17.9	4.8	+
	5 (1982)	SWWV5	E	61	26.1	22.3	38.2	1.7	23.6	23.5	43.3	1.8	+
	6 (1983)	SWWV6	E	11	4.5	5.1	2.9	0.6	3.1	5.5	2.8	0.9	0

continued...

TABLE 100. (CONT'D)

	''Exposure'' infection episode			Infection incidence[a]		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable	Jointly independent episode group[b]	No.	%	Low (<3) IR	High ($\geq$3) IR	AEI Group RR	Low (<1) IR	Intermediate IR	High (>5) IR	AEI Level RR	Risk ratio score[c]
SNV (All serum neutralization viruses)													
	1 (Spr 82)	SSNV1		20	10.9	9.8	13.1	1.3	10.9	11.4	8.7	0.8	0
	2 (Sum 82)	SSNV2		22	11.6	11.3	12.5	1.1	6.3	12.5	21.7	3.4	0
	5 (1982)	SSNV5		81	36.0	33.9	42.6	1.3	31.0	36.3	46.7	1.5	0
	3 (Spr 83)	SSNV3	D	12	5.7	7.3	2.7	0.4	7.3	5.5	3.3	0.5	–
	4 (Sum 83)	SSNV4	D	29	13.9	14.4	12.2	0.9	8.3	16.8	13.3	1.6	0
	6 (1983)	SSNV6		47	21.3	21.0	21.9	1.0	18.0	21.4	26.5	1.5	0

a Based on all observed individuals for whom an AEI exposure estimate was available.

b Classification criteria for the jointly independent groups of exposure infection episodes were given in Table 15.

c Risk ratio score criteria were given in Table 17.

TABLE 101. INFECTION INCIDENCE RATES[a] BY EXPOSURE GROUPS AND LEVELS AND RISK RATIO SCORE OF INFECTION EPISODES CLASSIFIED AS CONTROL SITUATIONS

''Control'' infection episode			Jointly independent episode group[b]	Infection incidence[a]		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							Risk ratio score[c]
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable		No.	%	Low (<3) IR	High ($\geq$3) IR	AEI Group RR	Low (<1) IR	Intermediate IR	High (>5) IR	AEI Level RR	
Clinical (C)													
VIR (Viruses, excluding adeno and immunization polio)													
	8 (Sum 80)	CVIR8	C	12	42.9	35.7	62.5	1.8	43	54	(0)	-	0
	9 (Sum 81)	CVIR9	C	9	31.0	34.8	16.7	0.5	60	18	(0)	-	--
Serologic (S)													
AD3 (Adeno 3)													
	Baseline	SAD30	C	13	5.1	6.1	3.3	0.5	3.3	6.8	0	0	0
AD5 (Adeno 5)													
	Baseline	SAD50	C	7	2.8	2.5	3.4	1.4	3.2	3.4	0	0	0
AD7 (Adeno 7)													
	Baseline	SAD70	C	6	2.0	1.0	3.8	3.8	1.2	1.6	5.1	4.2	++
CB2 (Coxsackie B2)													
	Baseline	SCB20	C	14	5.7	6.4	4.5	0.7	11.9	3.3	5.7	0.5	0
CB4 (Coxsackie B4)													
	Baseline	SCB40	C	16	6.6	6.4	6.9	1.1	8.5	7.4	0	0	0
CB5 (Coxsackie B5)													
	Baseline	SCB50	C	11	3.9	2.7	6.4	2.4	1.3	4.8	5.3	4.2	+
E01 (Echo 1)													
	Baseline	SE010	C	7	2.4	2.1	3.1	1.5	1.3	3.5	0	0	0
E03 (Echo 3)													
	Baseline	SE030	C	12	4.6	5.5	3.2	0.6	11.9	2.4	2.8	0.2	-
E09 (Echo 9)													
	Baseline	SE090	C	8	3.0	1.1	6.4	5.6	2.8	2.5	5.4	1.9	+
E11 (Echo 11)													
	Baseline	SE110	C	17	6.0	5.3	7.4	1.4	9.1	4.6	5.7	0.6	0

continued...

TABLE 101. (CONT'D)

''Control'' infection episode			Jointly independent episode group^b	Infection incidence^a		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							Risk ratio score^c
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable		No.	%	Low (<3) IR	High (≥3) IR	AEI Group RR	Low (<1) IR	Inter- mediate IR	High (>5) IR	AEI Level RR	
E20 (Echo 20)													
	Baseline	SE200	C	5	1.9	2.3	1.0	0.4	1.5	2.4	0	0	0
E24 (Echo 24)													
	Baseline	SE240	C	8	3.0	2.9	3.1	1.0	3.1	3.0	2.8	0.9	0
PL1 (Polio 1)													
	Baseline	SPL10		69	28.4	28.0	29.0	1.0	22.6	28.1	37.8	1.7	0
	67 Salk immunized adults:		C	49	73.1	72	74	1.0	62	79	69	1.1	0
	34 Sabin immunized children:		C	17	43.6	46	36	0.8	33	44	60	1.8	0
PL2 (Polio 2)													
	Baseline	SPL20		72	30.0	28.4	32.6	1.1	26.9	29.8	35.1	1.3	0
	67 Salk immunized adults:		C	51	76.1	78	74	1.0	62	87	63	1.0	0
	33 Sabin immunized children:		C	19	50.0	48	55	1.1	67	42	60	0.9	0
PL3 (Polio 3)													
	Baseline	SPL30		71	29.7	26.5	34.8	1.3	25.5	25.8	51.4	2.0	0
	67 Salk immunized adults:		C	56	83.6	81	87	1.1	77	82	94	1.2	0
	32 Sabin immunized children:		C	15	40.5	38	45	1.2	38	33	80	2.1	0
RE1 (Reo 1)													
	Baseline	SRE10	C	35	12.5	15.6	6.3	0.4	22.7	8.9	8.1	0.4	-
RE2 (Reo 2)													
	Baseline	SRE20	C	37	13.4	14.4	11.5	0.8	12.7	12.4	19.4	1.5	0
ROT (Rotavirus)													
	Baseline	SROT0	C	11	36.7	30.8	41.2	1.3	-	33	44	Large	+
INA (Influenza A)													
	0 (1980-81)	SINA0	C	19	10.2	10.6	9.3	0.9	5.8	11.1	15.4	2.7	0
	1 (1981-82)	SINA1	C	6	2.6	2.5	2.8	1.1	1.6	3.7	0	0	0
	3 (1982-83)	SINA3	C	35	13.8	15.2	11.1	0.7	14.3	14.4	10.5	0.7	0

continued...

TABLE 101 (CONT'D)

''Control'' infection episode			Jointly independent episode group[b]	Infection incidence[a]		Infection incidence rates (IR) and and risk ratios ($RR=IR_{Hi}/IR_{Lo}$), %							Risk ratio score[c]
						by two AEI groups			by three AEI levels				
Agent	Period of observation	Dependent variable		No.	%	Low (<3) IR	High (≥3) IR	AEI Group RR	Low (<1) IR	Intermediate IR	High (>5) IR	AEI Level RR	
POR (Sporadic serum neutralization viruses)													
	Baseline	SPORO	C	8	3.7	3.8	3.7	1.0	4.4	3.6	3.1	0.7	0
SNV (All serum neutralization viruses)													
	Baseline	SSNVO	F	98	47.1	44.9	51.4	1.1	57.4	44.9	37.0	0.6	0

a Based on all observed individuals for whom an AEI exposure estimate was available.

b Classification criteria for the jointly independent groups of control infection episodes were given in Table 15.

c Risk ratio score criteria were given in Table 17.

100 and 101 both for exposure groups and for exposure levels. The risk ratios vary widely, as expected for the low incidence of infections. About half of the group and level risk ratios for the control infection episodes in Table 101 exceed 1.0, as expected. However, a large majority (about 2/3) of both the group and level risk ratios for the exposure infection episodes in Table 100 exceed 1.0. Since this suggests a potential correlation of infections with wastewater aerosol exposure in exposure infection episodes, this phenomenon is investigated more carefully below.

Evaluation of Association of Infections with Aerosol Exposure via Risk Ratio Scores

A risk ratio score was assigned to each infection episode observed in the LISS as described in Section 4J. The risk ratio score criteria were symmetric with regard to the high and low exposure groups and levels (i.e., an infection pattern that would be scored + if the excess infections occurred in the high exposure group and level, would be scored - if the equivalent excess infections occurred in the low group and level). Thus, in the absence of any effect, random variation should produce an equal number of positive and negative risk ratio scores.

The assigned risk ratio scores are presented in Tables 100 and 101. A preponderance of positive (+ or ++) scores over negative (- and - -) scores is seen for the exposure situations (see Table 100), but not for the control situations (see Table 101).

The distribution of risk ratio scores obtained for each group of independent infection episodes was analyzed to provide a sensitive overview of any apparent association of infection events with wastewater aerosol exposure. The criteria for six mutually exclusive and jointly independent groups of episodes were presented in Table 15. The infection episodes placed in each of these groups are shown in Tables 100 and 101. The frequency with which each risk ratio score occurred was determined for all six groups of independent episodes. The frequency distributions are presented in Table 102. If aerosol exposure had no effect on infections, one would expect random variations to produce a symmetric distribution of risk ratio scores about 0, with approximately equal numbers of positive and negative scores and of ++ and - - scores. Symmetry would be expected because of the symmetric treatment of ''high'' and ''low'' exposure groups and levels in the risk ratio criteria. A one-sided sign test of the number of positive scores (++ or +) compared to the number of negative scores (- - or -) was conducted for each jointly independent group (see lower portion of Table 102), to determine if there was a significant excess of positive risk ratio scores for the infection episodes in the group.

Let us first consider the findings in Table 102 from the risk ratio scores for infection episodes to single or sporadic agents (Groups A, B and C). The frequency distribution of risk ratio scores for the control infection episodes (Group C) were symmetric about 0, in accord with our expectation for this group. However, among the exposure infection episodes occurring in single seasons (Group A), there were nine episodes with positive risk ratio scores, but none with negative scores. This excess of positive

TABLE 102. SIGNIFICANCE OF FREQUENCY DISTRIBUTIONS OF RISK RATIO SCORES BY GROUP[a] OF JOINTLY INDEPENDENT INFECTION EPISODES

FREQUENCY DISTRIBUTIONS OF RISK RATIO SCORES

	Single and sporadic agent infection episodes			Grouped agent infection episodes		
	Exposure situations		Control situations	Exposure situations		Control situations
Risk ratio score	Group A (single seasons)	Group B (years)	Group C (baseline+ influenza)	Group D (single seasons)	Group E (years)	Group F (baseline)
--	0	0	1	0	0	0
-	0	2	2	1	0	0
0	22	10	20	5	1	1
+	7	7	3	1	1	0
++	2	0	1	1	0	0
TOTAL	31	19	27	8	2	1

SIGNIFICANT EXCESS OF + (OR ++) RISK RATIO SCORES IN FREQUENCY DISTRIBUTION[b]

	Group A	Group B	Group C	Group D	Group E	Group F
Total negative scores (- or --)	0	2	3	1	0	0
Total postive scores (+ or ++)	9	7	4	2	1	0
Significant excess of positive scores? (p-value)[b]	Yes (0.002)	Maybe (0.09)	No	No	No	No

[a] See Tables 100 and 101 for episode assignment to jointly independent groups. See Table 15 for group classification criteria.

[b] One-sided sign test of total positive scores vs. total negative scores.

scores was highly significant (p=0.002). Among exposure episodes of 1-year duration (Group B), there were seven positive RR scores versus two negative scores. The excess of positive scores in Group B approaches significance (p=0.09), considering the smaller number of infection episodes in Group B. The RR score results for single and sporadic agent episodes of infection suggest that an excess risk of infection was associated with wastewater aerosol exposure.

The observation periods in which the Group A exposure infection episodes with positive RR scores occurred was (see Table 100):

Spring 1982 - 3 episodes (PL1 immunized, PL1 not immunized, PL2 immunized)
Summer 1982 - 4 episodes (VIR, CB4, CB5, ROT)
Spring 1983 - 0 episodes
Summer 1983 - 2 episodes (KLB, E24)

This seasonable distribution is consistent with the hypothesis of association of viral infections with wastewater aerosol exposure. The relative aerosol exposure measure to enteroviruses and indicator organisms from the wastewater spray irrigation was greater in the 1982 irrigation periods, especially summer 1982, and lowest in the spring 1983 period (compare RAEM for enteroviruses by irrigation period in Table 42). (Since poliovirus seroconversions were investigated only for the spring 1982 irrigation period, it was not possible to observe additional polio infection episodes in later seasons.) Thus, the seasonal distribution of Group A episodes with positive RR scores is correlated with seasonal microorganism (especially enteroviruses) aerosol exposure from wastewater spray irrigation, suggesting a dose-response relationship.

The excess Group B exposure infection episodes with positive RR scores occurred both in 1983 (three excess positive episodes) and in 1982 (two excess positive episodes). The relative aerosol exposure measure data in Table 42 suggests greater aerosol exposure to enteroviruses and indicator organisms from spray irrigation in 1982 rather than in 1983. The excess Group B episodes with positive RR scores lack both statistical evidence of excess positive episodes and the dose-response pattern anticipated for wastewater irrigation effects.

There were fewer independent infection episodes to groups of agents. Consequently, there were insufficient negative and positive RR scores by which to detect a significant excess of positive scores using the sign test. The only control infection episode (Group F) had no distinct exposure pattern of infection incidence rates (RR score=0). The independent single season exposure episodes to grouped agents (Group D) had a fairly symmetric distribution of RR scores about 0, with one excess positive score. The positive score episodes in Group D occurred in summer 1982 and summer 1983, while the negative score episode occurred in spring 1983. One of the two Group E exposure infection episodes had a positive risk ratio score; SWWV5 occurred in 1982. The results from the RR scores of independent grouped-agent infection episodes (Groups D-F) are consistent with the findings for the single agents (Groups A-C). Accumulation of the single agent episodes with RR scores of 0 in the grouped agent episodes and the smaller number of grouped agent episodes may have reduced the sensitivity of the distribution of the RR score method to detect wastewater irrigation effects in the grouped-agent episodes.

L. STATISTICAL ANALYSIS

The standard statistical analyses of infection episodes were performed in three major stages:

1) Preliminary Analysis--comparison of the low exposure group (AEI<3) and the high exposure group (AEI≥3) with respect to individual and household characteristics in order to determine if the two exposure groups differed significantly with regard to these factors

2) Confirmatory Analysis--comparison of infection rates in exposure groups to determine the presence of any association of infection and wastewater aerosol exposure

3) Exploratory Analysis--investigation of whether the presence of infection was associated with a set of potential predictor variables, and in particular with the degree of aerosol exposure.

Preliminary Analysis

Prior to conducting tests for association of infection rates and exposure, the exposure groups were compared with respect to other characteristics which could influence the outcome of these tests. In the high and low exposure groups, the proportion in each category of a characteristic was calculated. A standard chi-square test for equality of proportions (or Fisher's exact test) was done for each characteristic in each population (fecal donors and blood donors) in the six seasons of data plus a baseline data set. The fecal donor and blood donor populations were defined for each season as those individuals or households donating the necessary series of specimens to determine the infection status (see Table 43). Characteristics which were known to be constant over a household were tested using the household as the unit of observation. Household exposure to wastewater aerosols was defined as the maximum participant exposure level observed in the household. The results of these comparisons of exposure groups are given in Tables 103 through 107 for most individual and household characteristics, in Table 108 for previous titer, and in Table 109 for eating at local restaurants. Both the percentage in each category of the variable and the range of the probability value for each test are shown in the tables. The exposure groups tended to differ in certain characteristics on a seasonal basis (i.e., in both spring seasons or in both summer seasons) because many residents in the middle of Wilson shifted exposure groups by season due to seasonal differences in the prevailing wind direction.

For these tables, a judgment was made about the variable(s) to be used for stratification prior to comparison of infection rates in exposure groups. The relative importance, consistency and magnitude of differences across seasons and quality of the data for each variable were considered. To ensure consistency, a variable was considered for use as a stratifying

TABLE 103. COMPARISON OF EXPOSURE GROUPS WITH RESPECT TO HOUSEHOLD CHARACTERISTICS BY BASELINE AND IRRIGATION SEASON--BLOOD DONORS

(Entries are percent of households with each characteristic in each exposure group)

	Baseline[b]			Spring[c] 1982			Summer[d] 1982			Spring[e] 1983			Summer[f] 1983		
	exposure			exposure			exposure			exposure			exposure		
Characteristic	p[a]	Low	High	p	Low	High	p	Low	High	p	Low	High	p	Low	High
Number of Households		72	56		69	59		86	41		60	53		78	31
Race															
% caucasian		83	82		83	83		83	81		80	87		81	90
% hispanic		17	18		17	17		17	19		20	13		19	10
Household Size															
% 1-2 members		53	63		57	61		55	66		52	64		56	64
% 3-4 members		28	23		23	24		25	19		27	21		23	23
% 5+ members		19	14		20	15		20	15		21	15		21	13
Head of Household Education													*		
% 0-11 years		48	43		50	42		48	40		50	34		47	29
% 12 years		31	32		29	32		27	43		27	40		26	55
% 13+ years		21	25		21	26		26	17		23	26		27	16
Most Educated Family Member															
% 0-11 years		18	11		18	11		19	6		17	14		18	10
% 12 years		28	33		30	34		30	36		24	39		27	42
% 13+ years		54	56		52	55		51	58		59	47		55	48
Head of Household Occupation							*						+		
% professional or manager		18	12		16	14		19	10		18	15		18	13
% farmer		32	43		33	42		30	53		35	42		32	55
% other		50	45		51	44		51	37		47	43		50	32
Income, in 1979															
% less than $9,999		38	44		38	44		41	35		36	39		36	40
% $10,000-$19,999		22	28		24	26		25	27		24	29		26	23
% $20,000-$29,999		13	15		12	18		13	20		13	19		16	17
% $30,000+		27	13		26	12		21	18		27	13		22	20
Air Conditioning System										+			**		
% none		13	17		11	16		12	16		12	11		12	10
% refrigeration		53	36		55	37		52	31		57	37		56	26
% evaporative cooler		34	47		34	47		36	53		31	52		32	64

continued...

TABLE 103. (CONT'D)

	Baseline[b] exposure			Spring[c] 1982 exposure			Summer[d] 1982 exposure			Spring[e] 1983 exposure			Summer[f] 1983 exposure		
Characteristic	p[a]	Low	High	p	Low	High	p	Low	High	p	Low	High	p	Low	High
Use of Air Conditioning															
% all or most of time		32	40		32	38		31	43		35	42	na	38	42
% some each day		17	13		19	16		20	15		17	13		17	6
% only when very hot		39	31		38	31		36	27		37	32		33	39
% never		12	16		11	15		13	15		11	13		12	13
Drinking Water Supply	**			**						*					
% private well		63	36		61	36		49	46		60	40		50	52
% public supply		37	64		39	64		51	54		40	60		50	48

a Blank if $p>0.10$, + if $0.05<p\leq0.10$, * if $0.01<p\leq0.05$, ** if $0.001<p\leq0.01$, *** if $p\leq0.001$, na if chi-square test not done due to low expected frequencies.

b Baseline period of observation: Jun 1980–Jan 1982; exposure based on AEI for Spring 1982 irrigation period

c Spring 1982 period of observation: Jan–Jun 1982

d Summer 1982 period of observation: Jun–Dec 1982

e Spring 1983 period of observation: Dec 1982–Oct 1983

f Summer 1983 period of observation: Jun–Oct 1983

TABLE 104. COMPARISON OF EXPOSURE GROUPS WITH RESPECT TO HOUSEHOLD CHARACTERISTICS BY BASELINE AND IRRIGATION YEAR--BLOOD DONORS
(Entries are percent of households with each characteristic in each exposure group)

	Baseline[b] exposure			1982[c] exposure			1983[d] exposure		
Characteristic	p[a]	Low	High	p	Low	High	p	Low	High
Number of Households		72	56		84	44		72	40
Race									
% caucasian		83	82		83	82		81	90
% hispanic		17	18		17	18		19	10
Household Size									
% 1-2 members		53	63		56	64		53	68
% 3-4 members		28	23		24	23		26	20
% 5+ members		19	14		20	13		21	12
Head of Household Education									
% 0-11 years		48	43		50	41		47	32
% 12 years		31	32		25	41		28	43
% 13+ years		21	25		25	18		25	25
Most Educated Family Member									
% 0-11 years		18	11		17	9		17	12
% 12 years		28	33		32	32		31	33
% 13+ years		54	56		51	59		52	55
Head of Household Occupation				*			*		
% professional or manager		18	12		17	12		18	15
% farmer		32	43		30	52		28	55
% other		50	45		53	36		54	30
Income, in 1979									
% less than $9,999		38	44		42	39		34	41
% $10,000-$19,999		22	28		24	26		29	21
% $20,000-$29,999		13	15		11	21		13	23
% $30,000+		27	13		23	14		24	15
Air Conditioning System				+					
% none		13	17		13	14		12	12
% refrigeration		53	36		54	32		54	35
% evaporative cooler		34	47		33	54		34	53
Use of Air Conditioning									
% all or most of time		32	40		31	42		33	48
% some each day		17	13		20	11		21	5
% only when very hot		39	31		36	33		35	32
% never		12	16		13	14		11	15
Drinking Water Supply	**								
% private well		63	36		50	48		51	47
% public supply		37	64		50	52		49	53

a Blank if $p>0.10$, + if $0.05<p\leq0.10$, * if $0.01<p\leq0.05$, ** if $0.001<p\leq0.01$, *** if $p\leq0.001$ in chi-square test.

b Baseline period of observation: Jan 1980-Jan 1982.

c 1982 period of observation: Jan-Dec 1982.

d 1983 period of observation: Dec 1982-Oct 1983.

TABLE 105. COMPARISON OF EXPOSURE GROUPS WITH RESPECT TO HOUSEHOLD CHARACTERISTICS--FECAL DONORS
(Entries are percent of households with each characteristic in each exposure group)

	Baseline^{b} exposure			Spring^{c} 1982 exposure			Summer^{d} 1982 exposure			Spring^{e} 1983 exposure			Summer^{f} 1983 exposure		
Characteristic	p^{a}	Low	High	p	Low	High	p	Low	High	p	Low	High	p	Low	High
Number of Households		21	10		52	41		60	24		40	42		55	24
Race															
% caucasian		62	60		85	88		87	83		83	88		84	88
% hispanic		38	40		15	12		13	17		17	12		16	12
Household Size	na														
% 1-2 members		0	10		54	61		57	54		48	64		58	54
% 3-4 members		48	60		23	24		21	29		25	22		22	29
% 5+ members		52	30		23	15		22	17		27	14		20	17
Head of Household Education	na														
% 0-11 years		48	30		44	44		45	37		40	44		44	37
% 12 years		28	10		33	27		27	38		33	27		25	42
% 13+ years		24	60		23	29		28	25		27	29		31	21
Most Educated Family Member	na														
% 0-11 years		21	0		16	11		18	4		16	17		19	12
% 12 years		36	25		30	30		28	31		24	38		26	38
% 13+ years		43	75		54	59		54	65		60	45		55	50
Head of Household Occupation	na			+			***						*		
% professional or manager		29	30		23	14		23	8		22	10		18	8
% farmer		38	60		25	49		22	67		30	46		27	63
% other		33	10		52	37		55	25		48	44		55	29
Income, in 1979	na						na			+			na		
% less than $9,999		29	33		39	45		47	33		33	49		41	48
% $10,000-$19,999		19	45		20	22		19	29		21	24		22	22
% $20,000-$29,999		19	11		16	23		14	25		13	17		17	17
% $30,000		33	11		25	10		20	13		33	10		20	13
Air Conditioning System	na						*			**			***		
% none		29	22		16	16		16	9		16	15		8	8
% refrigeration		43	45		55	37		53	30		60	29		56	21
% evaporative cooler		28	33		29	47		31	61		24	56		26	71

continued...

TABLE 105. (CONT'D)

Characteristic	Baseline[b] exposure			Spring[c] 1982 exposure			Summer[d] 1982 exposure			Spring[e] 1983 exposure			Summer[f] 1983 exposure		
	p[a]	Low	High	p	Low	High	p	Low	High	p	Low	High	p	Low	High
Use of Air Conditioning	na						na						na		
% all or most of the time		29	50		31	41		28	54		33	34		33	42
% some each day		24	0		17	12		20	8		17	14		16	4
% only when very hot		28	30		39	32		37	29		35	38		33	46
% never		19	20		13	15		15	9		15	14		18	8
Drinking Water Supply	*			*						+					
% private well		48	10		64	44		47	63		60	43		49	58
% public supply		52	90		36	56		53	37		40	57		51	42

[a] Blank if $p>0.10$, + if $0.05<p\leq0.10$, * if $0.01<p\leq0.05$, ** if $0.001<p\leq0.01$, *** if $p\leq0.001$, na if chi-square test not done due to low expected frequencies.

[b] Baseline period of observation: 6/1-9/2/81.

[c] Spring 1982 period of observation: 1/4-4/2/82.

[d] Summer 1982 period of observation: 6/7-9/17/82.

[e] Spring 1983 period of observation: 1/31-4/22/83.

[f] Summer 1983 period of observation: 6/6-8/19/83.

TABLE 106. COMPARISON OF EXPOSURE GROUPS WITH RESPECT TO INDIVIDUAL CHARACTERISTICS—BLOOD DONORS

(Entries are percent of individuals with each characteristic in each exposure group)

	Spring 1982			Summer 1982			Spring 1983			Summer 1983		
		Exposure			Exposure			Exposure			Exposure	
Characteristic	p^a	Low	High	p	Low	High	p	Low	High	p	Low	High
Number of individuals		203	118		247	69		181	103		207	58
Age group												
% 0-5 years		2	6		5	4		3	9		5	7
% 6-17 years		27	24		28	23		28	26		27	24
% 18-44 years		32	30		30	30		29	27		27	33
% 45-64 years		25	24		24	28		27	23		28	26
% 65+ years		14	16		13	15		13	15		14	10
Gender												
% male		47	48		47	51		46	49		46	48
% female		53	52		53	49		54	51		54	52
Tap water consumed—vs. others your age												
% less than average		12	19		14	21		11	20		14	19
% average		71	71		73	60		74	67		73	64
% more than average		17	10		13	19		15	13		13	17
Time spent in Lubbock												
% 0-1 hours/week		36	36		33	37		35	35		35	33
% 2-11 hours/week		45	47		46	48		46	51		42	48
% 12+ hours/week		19	17		21	15		19	14		23	19
Contacts per week with ≥10 people												
% 0-5 contacts		41	42		40	54		40	47		42	54
% 6-10 contacts		34	29		33	27		35	26		34	25
% 11+ contacts		25	29		27	19		25	27		25	21
Smokes cigarettes regularly												
% no		90	85		88	88		90	86		87	90
% yes		10	15		12	12		10	14		13	10

continued...

TABLE 106. (CONT'D)

	Spring 1982			Summer 1982			Spring 1983			Summer 1983		
		Exposure			Exposure			Exposure			Exposure	
Characteristic	p[a]	Low	High	p	Low	High	p	Low	High	p	Low	High
Smokes cigarettes regularly 1983												
% no		88	87		88	87		88	88		88	89
% yes		12	13		12	13		12	12		12	11
Chews tobacco regularly				**			*			*		
% no		94	88		95	83		95	88		94	86
% yes		6	12		5	17		5	12		6	14
Any respiratory illness												
% no		73	72		74	71		75	70		86	67
% yes		27	28		26	29		25	30		24	33
Ever had pneumonia												
% no		91	94		92	93		92	92		91	95
% yes		9	6		8	7		8	8		9	5
Any heart condition												
% no		79	77		81	72		80	77		80	78
% yes		21	23		19	28		20	23		20	22
Any abdominal condition												
% no		84	81		85	80		85	82		82	84
% yes		16	19		15	20		15	18		18	16
Any other condition												
% no		69	67		70	64		69	68		68	71
% yes		31	33		30	36		31	32		32	29
Polio immunization	**											
% no		84	70									
% yes		16	30									

[a] Blank if $p>0.10$, * if $0.01<p\leq 0.05$, ** if $0.001<p\leq 0.01$ in chi-square or Fisher's exact test.

TABLE 107. COMPARISON OF EXPOSURE GROUPS WITH RESPECT TO INDIVIDUAL CHARACTERISTICS—FECAL DONORS

(Entries are percent of individuals with each characteristic in each exposure group)

	Spring 1982			Summer 1982			Spring 1983			Summer 1983		
		Exposure			Exposure			Exposure			Exposure	
Characteristic	p^a	Low	High	p	Low	High	p	Low	High	p	Low	High
Number of individuals		82	50		106	27		62	47		84	28
Age group												
% 0–5 years		12	16		14	15		11	15		14	14
% 6–17 years		32	20		29	18		26	15		23	18
% 18–44 years		17	20		21	26		15	21		12	25
% 45–64 years		21	24		18	30		32	26		31	29
% 65+ years		18	20		18	11		16	23		20	14
Gender												
% male		45	42		42	44		42	47		45	50
% female		55	58		58	56		58	53		55	50
Tap water consumed—vs. others your age				b			+					
% less than average		11	20		17	19		9	24		14	18
% average		71	67		69	62		69	58		68	64
% more than average		18	13		14	19		22	18		19	18
Time spent in Lubbock												
% 0-1 hours/week		26	40		24	26		23	30		23	29
% 2-11 hours/week		57	50		55	63		56	59		57	50
% 12+ hours/week		17	10		21	11		21	11		20	21
Contacts per week with ≥ 10 people	+			+			+					
% 0–5 contacts		36	50		39	62		38	59		45	52
% 6–10 contacts		41	20		36	15		38	23		34	26
% 11+ contacts		23	30		25	23		24	18		21	22
Smokes cigarettes regularly	+											
% no		98	90		93	93		92	94		95	93
% yes		2	10		7	7		8	6		5	7

continued...

TABLE 107. (CONT'D)

	Spring 1982			Summer 1982			Spring 1983			Summer 1983		
		Exposure			Exposure			Exposure			Exposure	
Characteristic	P[a]	Low	High	P	Low	High	P	Low	High	P	Low	High
Smokes Cigarettes Regularly 1983	+											
% no		95	87		90	88		93	93		96	89
% yes		5	13		10	12		7	7		4	11
Chews Tobacco				+								
% no		92	89		96	85		93	87		94	86
% yes		8	11		4	15		7	13		6	14
Any respiratory illness												
% no		80	76		73	70		77	70		75	71
% yes		20	24		27	30		23	30		25	29
Ever had pneumonia												
% no		95	92		93	89		94	91		92	96
% yes		5	8		7	11		6	9		8	4
Any heart conditions												
% no		80	68		81	70		79	66		74	71
% yes		20	32		19	30		21	34		26	29
Any abdominal conditions												
% no		87	80		86	78		84	79		82	86
% yes		13	20		14	22		16	21		18	14
Any other conditions												
% no		71	62		69	70		71	60		62	75
% yes		29	38		31	30		29	40		38	25

a Blank if p>0.10, + if 0.05<p≤0.10 in chi-square or Fisher's exact test.
b Chi-square test not done due to low expected frequency. Not feasible to collapse to 2x2 for Fisher's exact test.

Table 108. COMPARISON OF EXPOSURE GROUPS WITH RESPECT TO PREVIOUS TITER TO SEROLOGIC AGENTS
[Entries are number of individuals observed followed in parentheses by percent of individuals with previous titer below indicated titer level]

		Baseline[a] exposure			Spring 1982[b] exposure			Summer 1982[c] exposure			Spring 1983[d] exposure			Summer 1983[e] exposure			1982[f] exposure			1983[g] exposure		
Agent	Titer level	p[h]	Low	High	p	Low	High	p	Low	High	p	Low	High	p	Low	High	p	Low	High	p	Low	High
AD3	10		84(57)	57(63)														116(51)	38(53)			
AD5	10		81(57)	42(48)														109(49)	32(46)			
AD7	10		155(78)	84(81)																		
CB2	10		37(24)	21(24)														53(24)	17(24)			
CB4	10		48(31)	25(29)					54(24)	18(29)								50(22)	22(31)			
CB5	10		128(68)	63(67)		136(69)	67(61)		131(55)	34(50)					120(59)	39(68)		146(66)	50(71)		112(61)	45(63)
E01	10	+	178(92)	83(86)																		
E03	10		132(81)	72(76)											129(65)	39(72)	*	154(69)	59(82)		120(65)	50(70)
E09	10		111(63)	60(64)																		
E11	10		125(66)	56(60)		123(61)	57(53)		134(55)	39(57)					105(52)	32(57)		130(57)	42(58)		97(52)	40(56)
E19	10																+	169(77)	61(86)			
E20	10		148(86)	79(81)											151(79)	43(78)					140(78)	58(82)
E24	10		149(87)	88(90)											136(69)	42(78)		176(80)	57(79)		127(70)	55(79)
RE1	8		130(70)	65(68)		112(55)	65(59)															
RE2	8		87(48)	44(46)		69(35)	34(31)															
ROT	4		2(15)	2(12)		1(4)	1(4)		5(14)	2(11)		3(14)	1(4)		3(13)	3(14)		4(13)	2(10)		3(15)	1(4)
LEG[i]	64					84(49)	21(51)															
INA[j]	4		23(17)	5(9)	*	27(17)	4(6)					23(14)	8(9)									
PL1	4		22(15)	17(18)		6(4)	7(7)															
PL2	4		20(14)	15(16)		10(7)	8(8)															
PL3	4	+	60(41)	50(54)	*	21(14)	25(26)															

a Baseline titer: Jun 1980.
b Spring 1982 titer: Jan 1982.
c Summer 1982 titer: Jun 1982.
d Spring 1983 titer: Dec 1982.
e Summer 1983 titer: Jun 1983.
f 1982 titer: Jan 1982.
g 1983 titer: Dec 1982.
h Blank if $p>0.10$, + if $0.05<p\leq0.10$, * if $0.01<p\leq0.05$ in chi-square or Fisher's exact test.
i Titer Jun 1981.
j Periods of titer surveys: Jun 1980, Jun 1981, Jun 1982, respectively.

TABLE 109. COMPARISON OF EXPOSURE GROUPS WITH RESPECT TO FREQUENCY OF EATING FOOD PREPARED AT RESTAURANTS A AND B--FECAL DONORS
(Entries are percent of individuals with each frequency in each exposure group)

	Spring 1982 exposure			Summer 1982 exposure			Spring 1983 exposure			Summer 1983 exposure		
Characteristics	p	Low	High	p	Low	High	p	Low	High	p	Low	High
Number of Individuals		71	44		91	26		69	48		86	31
Restaurant A	**			***			***			***		
% ≥once/month		7	32		12	58		6	29		12	55
% <once/month		23	25		30	4		22	27		22	19
% never		70	43		58	38		72	44		66	26
Restaurant B												
% ≥once/month		15	16		22	19		17	17		21	23
% <once/month		27	16		13	12		25	19		15	13
% never		58	68		65	69		58	64		64	64

a Blank if $p > 0.10$, ** if $0.001 < p \leq 0.01$, *** if $p \leq 0.001$ in chi-square test.

variable if and only if 1) the variable was deemed to be epidemiologically important and 2) the hypothesis of equal proportions was rejected at the 0.01 level at least once or at the 0.05 level at least twice in the four irrigation seasons. If the variable met these criteria, stratification was used if the number of observations was large enough to permit statistical analysis in the stratified groups. While all of the variables listed in Tables 103 through 109 could have some individual or collective influence on infection rates, six variables were considered to be epidemiologically important enough to warrant stratification should they be imbalanced over exposure groups (Criterion 1). These were household size, head of household occupation, age, gender, previous titer for serological variables, and immunization status for polioviruses.

In the household-based analyses of the blood donor population (Tables 103 and 104), none of the variables met both criteria for stratification, i.e., neither household size nor head of household occupation met Criterion 2 for statistical significance. Note that in the summer 1982 and summer 1983 seasons, head of household occupation of the blood donors was near the criterion for statistical significance. This near-significant imbalance reflects the fact that the proportion of farmers in the high exposure group (53% in summer of 1982 and 55% in the summer of 1983) was greater than the proportion of farmers in the low exposure group (39% in the summer of 1982 and 32% in the summer of 1983). The hypothesis of equal proportions was rejected at the 0.01 level in the summer 1983 season for type of air conditioning system, because a majority (56%) of households in the low exposure group had refrigerated air conditioning while most (64%) of the households in the high exposure group had evaporative coolers. Also, drinking water supply was sufficiently different across exposure groups to be statistically significant at the 0.01 level in the baseline and spring 1982 seasons and at the 0.05 level in spring 1983. Although not significant, these proportions were sometimes reversed in the summer 1982 and summer 1983 seasons.

In the household-based analysis of the fecal donor population (Table 105), the high exposure group also contained significantly more farmers in summer 1982 and summer 1983 (67% and 63%, respectively, as shown by the head of household occupation variable) than the low exposure group (25% and 29%). Although this variable meets both criteria for stratification, the number of households in the fecal donor population was not large enough to permit statistical analysis in stratified groups as discussed in Section 4J, Statistical Methods. The exposure groups were also imbalanced with respect to type of air conditioning system, with more households in the high exposure group having evaporative coolers.

Comparison of exposure groups with respect to individual characteristics in the blood donor and fecal donor populations are shown in Tables 106 and 107. Two of the Criterion 1 variables (age and gender) were not statistically significant in any season. The exposure groups were significantly imbalanced with respect to a third Criterion 1 variable, polio immunization status (p=0.005) in spring 1982, with a larger proportion immunized in the high exposure group. A larger proportion of individuals regularly chewed tobacco in the high than in the low exposure group (Table 106). This difference was significant at p=0.01, 0.05 and 0.05 in summer 1982, spring 1983 and summer 1983, respectively. Tobacco chewing represents a possible hand-to-mouth exposure factor.

An imbalance in previous titer levels of individuals in the exposure groups could bias the tests for association between infection rates and wastewater exposure if one exposure group was significantly less susceptible to the agent than the other exposure group. Table 108 shows the comparison of exposure groups with respect to previous titer to the serologic agents for which titer levels were measured. Two agents, influenza A in June 1981 and echovirus 3 in January 1982, showed imbalance at the 0.05 level in one season, and these did not meet the criteria outlined above as justification for stratification prior to the confirmatory analysis. The exposure groups were significantly imbalanced for previous titer to poliovirus 3 for both the baseline and the spring 1982 periods of observation.

The exposure groups were very significantly imbalanced with respect to frequency of eating food prepared at restaurant A (Table 109) among the fecal donors surveyed. Those individuals in the fecal donor population who were in the high exposure group ate significantly more often at restaurant A than individuals in the low exposure group. This gives an alternative explanation for infections (especially bacterial) which could have been transmitted in food handling. For this reason, eating food prepared at restaurant A was considered a possible alternative explanation whenever a positive statistical association between infection rates and wastewater exposure is found, because this could negate the implication of the apparent association with wastewater exposure. The number of observations is too small for stratification into groups with respect to frequency of eating food prepared at the restaurant. Therefore, patronage of restaurant A was explored by logistic regression as an alternative explanation whenever an apparent association between infections and wastewater exposure was found (especially when they were bacterial infections).

In conclusion, when comparing infection rates in exposure groups, stratification on household or individual characteristics was done only for polioviruses on polio immunization status. However, all of these individual and household variables were considered in the exploratory logistic regression analyses of infections on degree of aerosol exposure and other potential predictor variables.

Confirmatory Analysis

Fisher's exact test was used to test the hypothesis that the incidence rates within the low and high exposure groups were equal for each agent in each irrigation season, with the one-sided alternative being that the high exposure group had a larger incidence rate than the low exposure group. One of the major requirements for the validity of this test is that the infections occurred independently in individuals. An individual could become infected either from the wastewater (primary exposure) or from another household member (secondary exposure). If secondary infections occurred frequently among members in large households, the validity of the statistical analysis could be questionable. Since there usually was more than one blood donor per household (and often more than one fecal donor), the independence of the responses was investigated. The data in Tables P-48 and P-49 in Appendix P showing the number of households by size with 0, 1 or 2 infections are not inconsistent with the hypothesis that the infections occurred independently. This can be seen from the fact that in only a few instances were there more than one seroconversion per household. Thus, it was concluded that the binomial was a suitable model for the occurrence of infections and that Fisher's exact test or a chi-square test for equality of the binomial proportions in the low and high exposure groups could be used.

In Tables 110 to 112, the incidence rates for bacterial, viral and serologic infections in the low (AEI<3) and high (AEI$\geq$3) wastewater aerosol exposure groups, were compared for the baseline period and for each of the four or six seasons of data. The study design specified that each individual be measured for serum titer and serologic infection status at the beginning of each season and at the end of each season. New infection events were defined in terms of seroconversions or changes in infection status. The serologic data did not permit inference as to whether the time of onset of observed serologic infection events was before, during or after the irrigation period for which association with aerosol exposure was being investigated. From the clinical data based on routine fecal specimens, it could be determined that the onset of many bacterial and viral infection events was during a period of irrigation (i.e., when the change in infection status occurred between two specimens donated during the irrigation period). Clinical infection status variables were constructed (denoted by ''-X'' in Tables 110 and 111) in which only the infection events with onset during an irrigation period were retained. For bacterial and viral infection events occurring between the fecal specimens collected prior to and shortly after an irrigation period commenced, it could not be determined whether the onset of the infection event preceded or followed

TABLE 110. COMPARISON OF INCIDENCE OF BACTERIAL INFECTIONS IN LOW AND HIGH EXPOSURE GROUPS

(Entries are number of infections observed followed by incidence rates expressed as percents (in parentheses), risk ratios (RR = high/low), and probability levels[a])

	Spring[b] 1982			Summer[c] 1982			Spring[d] 1983			Summer[e] 1983		
	exposure			exposure			exposure			exposure		
Agent	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR
KLB-X[f]	0	0	–	3(5)	2(10)	1.9	0	2(4)	–	3(5)	5(21)	4.5*
KLB-W[g]	2(3)	0	0	9(14)	4(17)	1.3	0	2(4)	–	5(8)	7(27)	3.6*
OOB-X	0	0	–	0	0	–	2(3)	3(6)	1.9	1(2)	0	0
OOB-W	0	0	–	1(2)	1(4)	2.7	2(3)	3(6)	1.9	2(3)	0	0
PBW-X	1(1)	1(2)	1.7	2(3)	1(4)	1.4	2(3)	0	0	0	0	–
PBW-W	2(3)	1(2)	0.8	2(3)	2(8)	2.7	2(3)	0	0	6(9)	3(12)	1.3

a Blank if $p>0.10$, * if $0.01<p\leq 0.05$ in Fisher's exact test.

b Spring 1982 period of observation: 1/4-4/2/82.

c Summer 1982 period of observation: 6/7-9/17/82.

d Spring 1983 period of observation: 1/31-4/22/83; X and W variables are the same for Spring 1983.

e Summer 1983 period of observation: 6/6-8/19/83.

f X: Onset of all infection events during irrigation period.

g W: Includes infection events for which onset may have preceded the irrigation period.

TABLE 111. COMPARISON OF INCIDENCE RATES OF VIRAL INFECTIONS IN LOW AND HIGH EXPOSURE GROUPS
(Entries are number of infections observed followed by incidence rates expressed as percents (in parentheses), risk ratios (RR = high/low), and probability levels[a])

Agent	Summer 1980[b] baseline exposure			Summer 1981[c] baseline exposure			Spring[d] 1982 exposure			Summer[e] 1982 exposure			Spring[f] 1983 exposure			Summer[g] 1983 exposure		
	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR
VIR-X[h]	5(36)	5(63)	1.8	6(35)	1(17)	0.5	6(8)	3(7)	0.9	6(8)	5(19)	2.5+	0	1(2)	—	1(1)	1(4)	2.8
VIR-W[i]	5(36)	5(63)	1.8	6(35)	1(17)	0.5	11(14)	4(9)	0.7	7(9)	5(19)	2.2	0	1(2)	—	4(6)	1(4)	0.7
WWI-X							4(6)	3(8)	1.2	8(14)	4(21)	1.6	2(3)	2(4)	1.3	3(5)	5(24)	4.8*
WWI-W							8(12)	4(10)	0.8	13(20)	7(32)	1.6	2(3)	2(4)	1.3	12(17)	10(38)	2.2*

a Blank if p>0.10, + if 0.05<p≤0.10, * if 0.01<p≤0.05 in Fisher's exact test.
b Sum 80 Baseline period of observation: 7/219/17/80. AEI for Spring 1982 irrigation period used to assess exposure association.
c Sum 81 Baseline period of observation: 6/1-9/2/81. AEI for Spring 1982 irrigation period used to assess exposure association.
d Spring 1982 period of observation: 1/4-4/2/82.
e Summer 1982 period of observation: 6/7-9/17/82.
f Spring 1983 period of observation: 1/31-4/22/83; X and W variables are the same for Spring 1983.
g Summer 1983 period of observation: 6/6-9/19/83.
h X: onset of all infection events during irrigation period.
i W: includes infection events for which onset may have preceded the irrigation period.

TABLE 112. COMPARISON OF INCIDENCE OF SEROLOGIC INFECTIONS IN LOW AND HIGH EXPOSURE GROUPS
[Entries are number of infections observed followed by incidence rates expressed as percents (in parentheses), risk ratios (RR = high/low), and probability levels[a]]

	Baseline[b] exposure			Spring[c] 1982 exposure			Summer[d] 1982 exposure			Spring[e] 1983 exposure			Summer[f] 1983 exposure			1982[g] exposure			1983[h] exposure		
Agent	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR	Low	High	RR
AD3	10(6)	3(3)	0.5	0	0	—	1(0)	0	0	1(1)	0	0	0	0	—	6(3)	1(1)	0.5	1(1)	0	0
AD5	4(3)	3(3)	1.4	3(2)	0	0	2(1)	0	0	2(1)	0	0	1(1)	0	0	8(4)	0	0	3(2)	1(1)	0.9
AD7	2(1)	4(4)	3.8	0	0	—	0	0	—	0	0	—	0	0	—	0	0	—	0	0	—
CB2	10(6)	4(5)	0.7	0	1(1)	—	1(0)	0	0							4(2)	5(7)	3.7*			
CB4	10(6)	6(7)	1.1	1(1)	2(2)	3.5	3(1)	2(3)	2.3							12(5)	6(8)	1.5			
CB5	5(3)	6(6)	2.4	2(1)	2(2)	1.8	2(1)	2(3)	3.3	1(1)	0	0	8(4)	0	0	5(2)	3(4)	1.8	8(4)	1(1)	0.3
E01	4(2)	3(3)	1.5	1(1)	0	0	0	0	—	0	0	—	0	0	—	1(0)	0	0	0	0	—
E03	9(5)	3(3)	0.6	0	0	—	3(1)	0	0	3(2)	0	0	8(4)	3(5)	1.3	8(4)	1(1)	0.4	11(6)	7(10)	1.7
E05	1(1)	2(2)	3.7	1(0)	0	0	0	0	—	0	0	—	0	0	—	1(0)	0	0	0	0	—
E09	2(1)	6(6)	5.6*	0	0	—	0	0	—	1(1)	0	0	1(1)	0	0	0	0	—	2(1)	0	0
E11	10(5)	7(7)	1.4	2(1)	2(2)	1.9	5(2)	2(3)	1.4	0	0	—	3(2)	3(5)	3.3	11(5)	8(11)	2.2+	5(3)	5(7)	2.6
E17	2(1)	0	0	1(1)	0	0	0	0	—	0	0	—	0	0	—	1(0)	0	0	2(1)	0	0
E19	2(1)	1(1)	0.9	0	0	—	0	1(1)	—	0	0	—	0	0	—	1(0)	2(3)	6.0	0	1(1)	—
E20	4(2)	1(1)	0.4	1(1)	0	0	1(0)	0	0	0	0	—	4(2)	2(4)	1.7	2(1)	0	0	7(4)	2(3)	0.7
E24	5(3)	3(3)	1.0	1(1)	1(1)	1.7	0	0	—	2(1)	2(2)	1.7	3(2)	4(7)	4.4*	6(3)	1(1)	0.5	4(2)	6(8)	3.9*
RE1	29(16)	6(6)	0.4	11(5)	5(5)	0.8				0	1(1)	—									
RE2	26(14)	11(11)	0.8	10(5)	3(3)	0.5				1(1)	1(1)	1.8									
ROT	4(31)	7(41)	1.3	1(4)	2(8)	2.0	1(3)	3(17)	6.0	1(5)	2(7)	1.6	3(13)	3(14)	1.1	3(9)	4(20)	2.1	4(20)	5(21)	1.0
INA[i]	14(11)	5(9)	0.9																		
INA[j]	4(2)	2(3)	1.1																		
INA[k]	25(15)	10(11)	0.7																		
LEG[l]				4(3)	2(3)	1.1															
POR	5(4)	3(4)	1.0	9(7)	4(6)	0.9	8(5)	1(2)	0.5							5(3)	0	0	8(5)	2(3)	0.6
WWV							8(5)	4(5)	1.0	10(5)	5(9)	1.7				40(22)	21(38)	1.7*	9(5)	2(3)	0.6
SNV	62(45)	36(51)	1.1	12(10)	8(13)	1.3	17(11)	5(13)	1.1	10(7)	2(3)	0.4	23(14)	6(12)	0.9	57(34)	23(43)	1.3	33(21)	14(22)	1.0

[a] Blank if $p>0.10$, + if $0.05<p\leq0.10$, * if $0.01<p\leq0.05$ in Fisher's exact test.
[b] Baseline period of observation: Jun 1980–Jan 1982.
[c] Spring 1982 period of observation: Jan–Jun 1982.
[d] Summer 1982 period of observation: Jun–Dec 1982.
[e] Spring 1983 period of observation: Dec 1982–Oct 1983.
[f] Summer 1983 period of observation: Jun–Oct 1983.
[g] 1982 period of observation: Jan–Dec 1982.
[h] 1983 period of observation: Dec 1982–Oct 1983.
[i] Period of observation: Jun 1980–Jun 1981.
[j] Period of observation: Jun 1981–Jun 1982.
[k] Period of observation: Jun 1982–Jun 1983.
[l] Period of observation: Jun 1981–Jun 1983.

the start of irrigation. The time of onset of these infection events was termed ''questionable,'' and the analysis was conducted with these observations included and again excluding these observations. Variables excluding and including these observations were denoted ''-X'' and ''-W'' in Tables 110 and 111. If the two analyses agreed, the result was accepted without change. If the two analyses disagreed, the result of the analysis excluding the questionable observations was accepted. Usually only a few infected individuals were in the questionable category as can be determined by comparing the entries in Tables 110 and 111; in all cases, the results using the X and W variables were similar.

Table 113 shows the incidence rates of poliovirus infections in the baseline and spring 1982 periods. Mantel-Haenszel tests were used to test for association between infection and wastewater exposure with the individuals stratified on immunization status.

TABLE 113. COMPARISON OF INCIDENCE OF POLIO INFECTIONS IN LOW AND HIGH EXPOSURE GROUPS STRATIFIED BY IMMUNIZATION STATUS
[Entries are number of infections observed followed by incidence rates expressed as percents (in parentheses), risk ratios (high/low), and probability levels[a] of the stratified Mantel-Haenszel (MH) test and Fisher's exact test]

	Immunization	Baseline[b] exposure				Spring 1982[c] exposure			
Agent	status	Low	High	RR	p	Low	High	RR	p
PL1	MH test								*
	Yes	39(61)	27(64)	1.1		1(4)	7(21)	5.9	*
	No	3(3)	0	-		2(2)	3(5)	2.9	
PL2	MH test								+
	Yes	41(65)	29(69)	1.1		1(4)	6(18)	5.1	+
	No	0	1(2)	-		1(1)	1(2)	2.0	
PL3	MH test								
	Yes	39(63)	32(76)	1.2		3(11)	4(12)	1.1	
	No	0	0	-		0	0	-	

a Blank if $p>0.10$, + if $0.05<p\leq 0.10$, * if $0.01<p\leq 0.05$ in the Mantel-Haenszel test controlling for polio immunization effect or in Fisher's exact test within immunization status strata.

b Baseline period of observation: Jun 1980-Jan 1982.

c Spring 1982 period of observation: Jan-Jun 1982.

Tests for association between incidence of infection and wastewater exposure were significant at the 0.05 level for seven organisms or organism groups in Tables 110 through 113.

- *Klebsiella* in summer 1983 at p=0.03 and 0.02 (KLB-X and -W),

- all wastewater isolates in summer 1983 at p=0.02 and 0.03 (WWI-X and -W),

- coxsackie B2 in 1982 at p=0.05 (CB2),

- echovirus 9 in baseline at p=0.02 (E09),

- echovirus 24 in summer 1983 and 1983 at p=0.05 and 0.03 (E24),

- all viruses in sprayed wastewater in 1982 at p=0.02 (WWV),

- poliovirus 1 in spring 1982 at p=0.02 (PL1).

Each of these agents was significant in only one season, since in the echo 24 case, summer 1983 infections are a subset of 1983 infections.

The possibility of false positive associations should be considered when interpreting these results. False positive associations are possible only when the infection incidence rate in the population is large enough to detect a difference between exposure groups. Thus, as recommended by Gart et al. (1979), the rate of false positives should be based only on independent infection episodes in which by definition the infection incidence rate of the population was large enough to possibly reject the null hypothesis. Gart et al. also point out that the expected rate of false positives in independent infection episodes is the average actual α-level. This will be considerably less than 5% when Fisher's exact test is used at α=0.05, since the cumulative distribution function of a discrete random variable is a step function which does not increase monotonically.

The actual rates of positive association in the six groups of independent infection episodes defined in Table 15 and identified in Tables 100 and 101 are presented in Table 114.

TABLE 114. RATE OF POSITIVE ASSOCIATIONS DETECTED BY THE STATISTICAL CONFIRMATORY ANALYSIS AT SIGNIFICANCE LEVEL 0.05 IN INDEPENDENT INFECTION EPISODES[a]

Independent episode group[b]	Number of independent episodes	No. of significant confirmatory analysis results ($p<0.05$)		Significant episodes
		No.	%	
A	31	2	6%	(CKLB4X, SPL11)
B	19	2	11%	(SCB25, SE246)
C (Control)	27	1	4%	(SE090)
D	8	1	13%	(CWWI4X)
E	2	1	(50%)	(SWWV5)
F (Control)	1	0	(0%)	

a From Tables 100 and 111.

b See Table 15.

In the 27 independent control infection episodes involving single and sporadic agents (Group C) which were tested, one spurious positive association for echovirus 9 was found (4% positive rate). Two of the 31 independent exposure episodes to single or sporadic agents spanning a single irrigation period were associated with wastewater aerosol exposure, a 6% positive rate for Group A. Of the 19 Group B exposure episodes to single or sporadic agents which spanned several irrigation periods, 2 (11%) were significantly associated with exposure. For independent infection episodes involving grouped agents, the rates of positive associations were 0/1 for the control episode, but 1/8=13% for single season exposure episodes (Group D) and 1/2=50% for year-long exposure episodes (Group E). The actual rate of positive association in control episodes was approximately equal to the expected false positive rate. In contrast, the actual rate of significant associations exceeded the false positive rate in each of the four independent groups of exposure episodes. The actual rate of positive associations in the exposure episodes appears to be at least twice as large as the false positive rate.

The possibility must also be recognized that important differences in incidence rates may exist, but were not detected by the statistical test. The probability of such a false negative result is determined by the true (and unknown) incidence rates in each of the two exposure groups and the number of individuals observed in each exposure group. In accord with intuition, the power of the test, that is, the probability of detecting a given difference in the two incidence rates p_1 (low exposure) and p_2 (high exposure), increases as the number of individuals increases. Further, the power to detect differences in p_1 and p_2 tends to increase as p_1 becomes very small. Table P-50 in Appendix P displays the actual sample sizes (n_1 in the low exposure group, n_2 in the high exposure group) and $\hat{p}_1$, the observed incidence rates in the low exposure group. It is then assumed that $p_1=\hat{p}_1$ and $p_2=p_1 + \Delta$ (where $\Delta = 0.05, 0.07, 0.10, 0.15, 0.20$ or 0.25). With n_1, n_2, p_1 and p_2 thus specified, the power of the test for which $\alpha=0.05$ is calculated and displayed in the body of Table P-50. This shows that in most cases only relatively large differences in p_1 and p_2 can be detected from these data and these statistical procedures with a power of 0.90 or greater. This means that the lack of a significant test result in a given instance could result either from the absence of important differences in p_1 and p_2 or the lack of power to detect a difference which is in fact present.

In conclusion, an excess of statistically significant associations of the presence of infection with wastewater aerosol exposure was found in the confirmatory analysis. The interpretation of the epidemiological importance of these significant associations must be moderated by recognition of the possibility that some of the tests may be significant only by chance and that some imbalances in the two populations may provide alternate explanations for the observed differences. On the other hand, the number of detected increases in incidence rates associated with the wastewater irrigation may be underestimated, considering the relatively modest power of the tests to detect small differences. The certainty of the results is also lessened when the observational nature of the study and the difficulty inherent in determining appropriate assignment of individuals to the exposure groups are considered.

Exploratory Logistic Regression Analysis

The exploratory logistic regression analysis investigated whether the presence of infection was associated with a set of potential predictor variables, and in particular with AEI, the degree of aerosol exposure. An analysis was performed for each infection episode in which there was a higher rate of infection in the high exposure group than in the low exposure group and in the high exposure level than in the low and intermediate exposure levels.

The effects of each predictor variable added in a stepwise manner to the logistic model were assessed by means of a maximum-likelihood-ratio chi-square test of the hypothesis that the explanatory power of that variable was zero. The goodness-of-fit of the devised models in describing the relationship between the probability of infection and the selected predictor variables was assessed using a test developed by Hosmer and Lemeshow (1980). A small p-value (e.g., $p<0.10$) indicates that the prediction equation does not fit the data.

For each constructed model, approximate 90% confidence intervals were obtained for the odds ratio. If the constructed confidence interval contained the value 1 it was concluded that the odds of having an infection were the same for the various categories of the predictor variable.

Four different analyses were performed in order to analyze the relationship between rate of infection and the chosen predictor variables. These four analyses are described below.

Analysis 1: Basic Analysis--

A stepwise logistic regression was performed to investigate whether the presence of infection was associated with a selected set of predictor variables. This analysis was repeated for each of the six seasons of data plus a baseline data set. The response variables used in each season are listed in Table 115 preceded by the previous titer predictor variable corresponding to each serologic single-agent response variable. The response and previous titer variables were described in more detail in Tables 97-99 and P-45 in Appendix P, respectively. Descriptions of the predictor variables under consideration are presented in Table 116. Table 117 lists the candidate set of predictor variables (besides previous titer) chosen from Table 116 for usage in the various stepwise regressions.

The restaurant variables and the alternative exposure variables were not included in the basic analysis. Since the restaurant variables were observed only for a small subset of the individuals, the investigation of a possible restaurant etiology was analyzed separately (see Analysis 2). Since the alternative exposure variables (FHRSEM, XDIREL and XDIREM) were highly correlated with AEI (see Table P-23 of Appendix P), a separate analysis of the route of wastewater exposure was performed (see Analysis 4) when AEI was found to be a significant variable. The polio immunization variables (IM1, SABINO, SALKO) were regressed only against the respective polio infection response variables (SPL11, SPL21 and SPL30).

TABLE 115. PREVIOUS TITER AND RESPONSE VARIABLES FOR LOGISTIC REGRESSION ANALYSES

Baseline 1980-81	Spring 1982	Summer 1982	Spring 1983	Summer 1983	Year 1982	Year 1983
CVIR8	CWWI1X	CPBW2X	COOB3	CKLB4X	PCB25[a]	PE036[a]
					SCB25	SE036
PAD70[a]	PROT1[a]	CPBW2W	CWWI3	CKLB4W		
SAD70	SROT1				PCB45[a]	PE116[a]
		CVIR2X	PROT3[a]	CWWI4X	SCB45	SE116
PCB50[a]	PPL11[a]		SROT3			
SCB50	SPL11	CVIR2W		CWWI4W	PCB55[a]	PE246[a]
					SCB55	SE246
PE090[a]	PPL21[a]	CWWI2X		PE034[a]		
SE090	SPL21			SE034	PE115[a]	SSNV6
		PCB42[a]			SE115	
PINA0[a]		SCB42		PE114[a]		
SINA0				SE114	PE195[a]	
		PCB52[a]			SE195	
PRE20[a]		SCB52		PE204[a]		
SRE20				SE204	PROT5[a]	
		PE112[a]			SROT5	
PROT0[a]		SE112		PE244[a]		
SROT0				SE244	SWWV5	
		PROT2[a]				
PPL30[a]		SROT2		PROT4[a]	SSNV5	
SPL30				SROT4		
		SWWV2				
		SSNV2				

[a] The log (base e) of these previous titer variables were used in the regression analyses.

Prefix
P Previous titer
C Clinical dependent variable
S Serologic dependent variable

TABLE 116. PREDICTOR VARIABLES FOR LOGISTIC REGRESSIONS[a]

Predictor variable	Code
1. AEI, Aerosol Exposure Index	See ''Aerosol Exposure Index'' in Section 4C
2. AGE82, age on June 30, 1982	Age, in years
3. SEX, sex	1 Male 2 Female
4. RESP, history of respiratory conditions	0 No 1 Yes
5. PNEU, history of pneumonia	0 No 1 Yes
6. HEART, history of heart conditions	0 No 1 Yes
7. ABDOM, history of gastrointestinal conditions	0 No 1 Yes
8. OTHERO, history of other chronic conditions	0 No 1 Yes
9. SMOKE/SMOKE3, current cigarette smoker	0 No 1 Yes
10. TCHEW, tobacco chewer in 1983	0 No 1 Yes
11. RACE, race	1 Caucasian 4 Hispanic
12. HHSIZGR, household size group	1 1-2 members 2 3-4 members 3 5 or more members
13. HOHEDGR, education group of household head	1 Grades 0-8 2 Grades 9-11 3 Grade 12 4 Some college 5 College graduate
14. HOHOCC, occupation of household head	1 Professional or manager 2 Farmer 3 Other
15. INCOME, income group (1979 family income)	1 <\$10,000 2 \$10,000-\$30,000 3 >\$30,000
16. ACSYS, type of air conditioning system	1 None 2 Refrigeration 3 Evaporative cooler
17. ACUSE, frequency of air conditioning use (in summer)	1 All or most of time 2 Some time each day 3 Only when very hot 4 Never or no air conditioning

continued...

TABLE 116. (CONT'D)

Predictor variable	Code
18. DWATER, drinking water source	1 Private well (rural) 2 Public supply (Wilson)
19. WCONSM, tap water consumed vs. others your age	1 Less than average 2 Average 3 More than average
20. CONTACT, contacts per week with ten or more people	1 Less than once 2 1 to 5 times 3 6 to 10 times 4 11 to 15 times 5 More than 15 times
21. TLUBOCK, time in Lubbock	Average hours per week spent in Lubbock
22. LNP__, natural logarithm of previous serologic titer to response variable agent	ln (previous titer)
23. RESTA, frequency ate food prepared at restaurant A	1 >Once/week 2 Once/week to once/month 3 <Once/month 4 Never
24. RESTB, frequency ate at restaurant B	1 >Once/week 2 Once/week to once/month 3 <Once/month 4 Never
25. FHRSEM, time on Hancock farm	Average hours per week spent on Hancock farm (see ''Additional Exposure Measures'' in Section 4C)
26. XDIREM, index of extensive direct wastewater contacts	See ''Additional Exposure Measures'' in Section 4C
27. XDIREL, level of direct wastewater contact	1 None (XDIREM=0) 2 Low (0.1≤XDIREM≤10) 3 High (XDIREM>10)
28. SALKO, Salk inactivated polio immunization in 1980-81	0 No 1 Yes
29. SABINO, Sabin oral polio immunization in 1980-81	0 No 1 Yes
30. IM1, polio immunization in spring 1982	0 No 1 Yes (Salk or Sabin)

a All predictor variables with more than two codes were treated as interval variables, except HOHOCC and ACSYS which were treated as categorical variables.

TABLE 117. PREDICTOR VARIABLES USED IN LOGISTIC REGRESSION ANALYSIS

Baseline	Spring 1982	Summer 1982	Spring 1983	Summer 1983	1982	1983
AEI	AEI	AEI	AEI	AEI	AEI	AEI
AGE82	AGE82	AGE82	AGE82	AGE82	AGE82	AGE82
SEX	SEX	SEX	SEX	SEX	SEX	SEX
RESP	RESP	RESP	RESP	RESP	RESP	RESP
PNEU	PNEU	PNEU	PNEU	PNEU	PNEU	PNEU
HEART	HEART	HEART	HEART	HEART	HEART	HEART
ABDOM	ABDOM	ABDOM	ABDOM	ABDOM	ABDOM	ABDOM
OTHERO	OTHERO	OTHERO	OTHERO	OTHERO	OTHERO	OTHERO
SMOKE	SMOKE3	SMOKE3	SMOKE3	SMOKE3	SMOKE3	SMOKE3
RACE	RACE	RACE	TCHEW	TCHEW	RACE	TCHEW
HHSIZGR	HHSIZGR	HHSIZGR	RACE	RACE	HHSIZGR	RACE
HOHEDGR	HOHEDGR	HOHEDGR	HHSIZGR	HHSIZGR	HOHEDGR	HHSIZGR
HOHOCC	HOHOCC	HOHOCC	HOHEDGR	HOHEDGR	HOHOCC	HOHEDGR
INCOME	INCOME	INCOME	HOHOCC	HOHOCC	INCOME	HOHOCC
ACUSE	ACUSE	ACUSE	INCOME	INCOME	ACUSE	INCOME
DWATER	DWATER	DWATER	ACSYS	ACSYS	DWATER	ACSYS
TLUBOCK	TLUBOCK	TLUBOCK	ACUSE	ACUSE	TLUBOCK	ACUSE
SALKO	IM1		DWATER	DWATER		DWATER
SABINO			WCONSM	WCONSM		WCONSM
			CONTACT	CONTACT		CONTACT
			TLUBOCK	TLUBOCK		TLUBOCK
RESTA	RESTA	RESTA	RESTA	RESTA	RESTA	RESTA
RESTB	RESTB	RESTB	RESTB	RESTB	RESTB	RESTB
FHRSEM	FHRSEM	FHRSEM	FHRSEM	FHRSEM	FHRSEM	FHRSEM
XDIREL	XDIREL	XDIREL	XDIREM	XDIREM	XDIREL	XDIREL

The results of the initial stepwise logistic regression runs are summarized in Tables 118 through 124 for each season of data. Included is a list of the tested agent, the significant predictor variables from the stepwise runs, approximate 90% confidence intervals on the odds ratios for the significant variables, and the p-value for the Hosmer goodness-of-fit test. Also given are the p-values from the chi-square test for the significance of the AEI variable both at the initial step (when AEI is the only variable in the equation) and at the final step (regardless of whether or not AEI entered the equation). Finally, indicated with each significant predictor variable is the category that had the higher infection rate.

A few observations, ranging up to 10% of the individuals observed per response variable, were deleted from each initial analysis because the values of certain predictor variables were missing. For those response variables providing good or marginal evidence of aerosol exposure association (see Tables 131 and 132), the basic analysis was rerun, deleting predictor variables with missing values when these variables were not significant in the initial analysis and estimating missing values of important variables where possible. These rerun analyses are presented in Table 125.

While controlling for the effects of significant monitored covariates, the logistic regression analysis identified four infection episodes in which the infections were associated with AEI at a final step p-value below 0.05:

- SE090--echovirus 9 in baseline (p=0.01)
- SPL11--poliovirus 1 in spring 1982 (p=0.01)
- SWWV2--seroconversions to wastewater isolates in summer 1982 (p=0.02)
- SSNV2--all seroconversions to serum neutralization-tested viruses in summer 1982 (p=0.04)

The significant covariates are presented in Table 125. The goodness-of-fit of each of these models was excellent.

The effect of excluding some of the observations in the initial runs can be seen by comparing the AEI significance p-values for the episodes in Table 125 with the same values for the initial run of the episode in Tables 118-124. When the excluded observations are influential, the effect can be major. This is illustrated by SCB42 with a p-value of 0.16 in Table 125 using all 289 observations, but a p-value of 0.01 in Table 120 for the run with one infected donor and 14 noninfected donors excluded. However, the effect on AEI significance of excluding some of the observations usually was minor (see SE090 and SE115, for example) or trivial (e.g., SPL11, CVIR2X and CKLB4X).

The poliovirus 1 infections in spring 1982 (SPL11) are shown in Table 125 to be significantly associated with three predictor variables: IM1--polio immunization in spring 1982, low LNPPL11--polio 1 antibody titer in January 1982, and high AEI--aerosol exposure in spring 1982. This infection episode was subsequently found to be the only episode consistently associated with

TABLE 118. LOGISTIC REGRESSION RESULTS FOR BASELINE INFECTION EPISODES

Agent	AEI Significance		Significant[c] predictor variable	90% Confidence interval for the odds ratio	Goodness of fit[d]
	Initial[a]	Final[b]			
CVIR8	$p>0.25$	$p>0.25$	AGE82 (young)	(0.68, 0.99)	0.79
SAD70	$p=0.12$	$p=0.23$	HOHEDGR (college educ. HOH)	(1.07, 3.13)	0.21
SCB50	$p>0.25$	$p>0.25$	LNPCB50 (high antibody level)	(1.07, 3.41)	0.48
SE090	$p=0.02$	$p=0.02$	AEI (high aerosol exposure)	(1.02,1.11)	0.38
SINA0	$p>0.25$	$p>0.25$	LNPINA0 (low antibody level) RACE (hispanics)	(0.25, 0.84) (1.02, 1.97)	0.11
SRE20	$p=0.12$	$p>0.25$	HOHOCC (farmer) HEART (heart history)	(1.38, 3.56) (1.04, 4.30)	0.96
SROT0	$p>0.25$	$p>0.25$	LNPROT0 (low antibody level) INCOME (high)	(0.23, 0.75) (1.04, 8.19)	0.63
SPL30	$p>0.25$	$p>0.25$	LNPPL30 (low antibody level) SALK0 (salk vaccination in Baseline) AGE82 (young) HOHEDGR (little educ. HOH)	(0.20, 0.69) (72.54, 1304.85) (0.92, 0.98) (0.50, 0.93)	0.45

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[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p\le.10$, then p-value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 119. LOGISTIC REGRESSION RESULTS FOR SPRING 1982 INFECTION EPISODES

Agent	AEI significance Initial[a]	Final[b]	Significant[c] predictor variable	90% Confidence interval for the odds ratio	Goodness of fit[d]
CWWI1X	$p>0.25$	$p>0.25$	INCOME (low)	(0.01, 0.53)	0.81
			RESP (respiratory history)	(2.83, 89.68)	
			HHSIZGR (small HH)	(0.08, 1.06)	
SROT1	$p>0.25$	$p>0.25$	OTHERO (history of other chronic conditions)	(2.86, 764.98)	0.65
			TLUBOCK (little time in Lubbock)	(0.55, 1.10)	
SPL11	$p=0.01$	$p=0.01$	IM1 (polio immunization in Spring 82)	(7.18, 101.98)	0.92
			LNPPL11 (low antibody level)	(0.14, 0.48)	
			AEI (high aerosol exposure)	(1.02, 1.10)	
SPL21	$p=0.11$	$p>0.25$	IM1 (polio immunization in Spring 82)	(6.56, 144.53)	0.31
			LNPPL21 (low antibody level)	(0.10, 0.47)	
			SEX (males)	(0.04, 0.93)	

[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p \leq .10$, then p-value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 120. LOGISTIC REGRESSION RESULTS FOR SUMMER 1982 INFECTION EPISODES

Agent	AEI significance		Significant[c] predictor variables	90% Confidence interval for for odds ratio	Goodness of fit[d]
	Initial[a]	Final[b]			
CPBW2X	p>0.25	p>0.25	AGE82 (elderly)	(0.99, 1.15)	0.05
CPBW2W	p>0.25	p>0.25	ABDOM (gastrointestinal history	(1.98, 98.98)	
CVIR2X	p=0.16	p=0.16	none		
CVIR2W	p=0.19	p=0.09	AGE82 (young)	(0.95, 0.99)	0.55
			AEI (high exposure)	(1.00, 1.04)	
CWWI2X	p>0.25	p>0.25	OTHERO (history of other chronic conditions)	(3.69, 78.35)	0.73
			DWATER (public water supply)	(2.82, 88.21)	
			ACUSE (regular A/C users)	(0.19, 0.80)	
SCB42	p>0.25	p=0.01	AGE82 (young)	(0.54, 0.92)	0.35
			HOHEDGR (college educ. HOH)	(1.15, 10.78)	
			SMOKE3 (current smoker)	(8.31, 1.25E7)	
			AEI (high exposure)	(1.01, 1.21)	
			RESP (respiratory history)	(0.86, 461.39)	
SCB52	p>0.25	p>0.25	none		
SE112	p=0.11	p=0.11	INCOME (low)	(0.14, 1.06)	0.25
SROT2	p>0.25	p>0.25	TLUBOCK (much time in Lubbock)	(1.01, 1.13)	0.44
SWwV2	p=0.04	p=0.05	AGE82 (young)	(0.93, 0.98)	0.58
			AEI (high exposure)	(1.00, 1.04)	
			INCOME (low)	(0.19, 0.79)	
			RACE (caucasians)	(0.40, 0.93)	
SSNV2	p=0.19	p=0.05	AGE82 (young)	(0.93, 0.98)	0.27
			INCOME (low)	(0.19, 0.71)	
			SMOKE3 (smoker)	(1.21, 13.74)	
			AEI (high exposure)	(1.01, 1.04)	
			DWATER (public water supply)	(1.16, 8.31)	
			RACE (caucasians)	(0.49, 0.99)	

[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p \leq .10$, then p value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 121. LOGISTIC REGRESSION RESULTS FOR SPRING 1983 INFECTION EPISODES

Agent	AEI significance		Significant[c] predictor variables	90% Confidence interval for the odds ratio	Goodness of fit[d]
	Initial[a]	Final[b]			
COOB3	p>0.25	p>0.25	none		
CWWI3	p>0.25	p>0.25	ABDOM (gastrointestinal history)	(2.89, 1.90E3)	0.36
			INCOME (low)	(0.02, 0.83)	
			TCHEW (tobacco chewers)	(1.39, 1.26E3)	
SROT3	p>0.25	p=0.21	HOHEDGR (college educ. HOH)	(0.91, 6.05)	0.79

[a] This is the p-value of AEI at the inital step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p \leq .10$, then p-value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 122. LOGISTIC REGRESSION RESULTS FOR SUMMER 1983 INFECTION EPISODES

Agent	AEI significance		Significant[c] predictor variables	90% Confidence interval for the odds ratio	Goodness of fit[d]
	Initial[a]	Final[b]			
CKLB4X	p=0.10	p=0.13	WCONSM (drinks a lot of water)	(1.08, 10.02)	0.16
CKLB4W	p=0.18	p=0.18	none		
CWWI4X	p=0.11	p=0.16	WCONSM (drinks a lot of water)	(1.11, 11.09)	0.12
CWWI4W	p>0.25	p>0.25	CONTACT (infrequent group contact)	(0.26, 0.75)	0.29
SE034	p>0.25	p>0.25	OTHERO (no history of other chronic condition)	(0.03, 1.01)	0.89
			SEX (females)	(1.10, 15.10)	
SE114	p>0.25	p>0.25	HHSIZGR (large HH)	(1.63, 42.94)	0.63
			DWATER (private wells)	(0.02, 0.82)	
SE204	p>0.25	p>0.25	HOHEDGR (college educ. HOH)	(1.23, 6.98)	0.07
			CONTACT (frequent group contacts)	(1.08, 4.19)	
			INCOME (high)	(0.90, 10.81)	
SE244	p>0.25	p>0.25	AGE82 (young)	(0.86, 0.97)	0.53
SROT4	p>0.25	p>0.25	LNPROT4 (low antibody level)	(0.07, 0.50)	0.50
			AGE82 (young)	(0.84, 1.03)	

[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation

[b] If $p \leq .10$, then p-value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 123. LOGISTIC REGRESSION RESULTS FOR 1982 INFECTION EPISODES

Agent	AEI significance		Significant[c] predictor variable	90% Confidence interval for the odds ratio	Goodness of fit[d]
	Initial[a]	Final[b]			
SCB25	p>0.25	p>0.25	SEX (males) AGE82 (elderly)	(0.02, 0.69) (1.00, 1.06)	0.74
SCB45	p>0.25	p>0.25	AGE82 (young)	(0.96, 0.99)	0.32
SCB55	p>0.25	p>0.25	AGE82 (young)	(0.92, 0.99)	0.39
SE115	p=0.04	p=0.06	RESP (no respiratory history) HOHOCC (farmer) DWATER (public water supply) AEI (high aerosol exposure)	(0.03, 1.06) (1.35, 5.18) (1.35, 9.35) (1.00, 1.03)	0.33
SE195	p>0.25	p>0.25	none		
SROT5	p>0.25	p>0.25	ACUSE (regular A/C users) HOHOCC (other occupation) HHSIZGR (small HH)	(0.01, 0.77) (1.31, 281.8) (0.03, 0.94)	0.95
SWWV5X	p=0.17	p>0.25	HOHOCC (farmer) PNEU (pneumonia history)	(1.23, 2.52) (1.04, 5.55)	0.83
SSNV5X	p>0.25	p>0.25	PNEU (pneumonia history) ACUSE (infrequent A/C user)	(1.81, 10.40) (1.06, 1.64)	0.84

[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p \leq .10$, then p-value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10 < p < 1.0$) indicates an acceptable fit.

TABLE 124. LOGISTIC REGRESSION RESULTS FOR 1983 INFECTION EPISODES

Agent	AEI significance Initial[a]	 Final[b]	Significant[c] predictor variables	90% Confidence interval for odds ratio	Goodness of fit[d]
SE036	p>0.25	p>0.25	LNPE036 (high antibody)	(1.21, 2.35)	0.56
			HEART (young)	(0.04, 1.14)	
SE116	p>0.25	p>0.25	HHSIZGR (large household)	(1.25, 5.96)	0.17
			DWATER (private wells)	(0.05, 0.65)	
SE246	p>0.25	p>0.25	AGE82 (young)	(0.91, 0.99)	0.17
			WCONSM (drinks little water)	(0.01, 0.31)	
			RACE (hispanics)	(1.69, 7.72)	
			HOHEDGR (college education, HOH)	(1.43, 4.93)	
SSNV6	p>0.25	p>0.25	ABDOM (No GI history)	(0.02, 0.60)	0.34
			SMOKE3 (nonsmoker)	(0.03, 0.80)	
			AGE82 (young)	(0.97, 0.99)	

[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p \le .10$, then p-value indicates X^2 to remove AFI at last step in model selection; otherwise p-value indicates X^2 to enter AFI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 125. RESULTS OF RERUN OF ANALYSIS 1 - INVESTIGATE INFECTION EPISODES WITH FEWER OBSERVATIONS DELETED

Season/Agent	AEI significance Initial[a]	 Final[b]	Significant[c] predictor variables	90% Confidence interval for the odds ratio	Goodness of fit[d]
BASELINE					
SE090	p=0.03	p=0.01	Race (hispanics)	(1.52, 4.16)	0.71
			AEI (high aerosol exposure	(1.02, 1.13)	
			RESP (respiratory history)	(1.37, 21.36)	
SPRING 1982					
SPL11	p=0.01	p=0.01	IM1 (polio immunization in Spring 1982)	(6.98, 98.18)	0.70
			LNPPL11 (low anti body level)	(0.14, 0.48)	
			AEI (high aerosol exposure)	(1.02, 1.10)	
SUMMER 1982					
CVIR2X	p=0.16	p=0.16	none		
CVIR2W	p=0.18	p=0.07	HHSIZGR (large household)	(1.14, 4.72)	0.53
			AEI (high aerosol exposure)	(1.00, 1.04)	
SCB42	p>0.25	p=0.16	AGE82 (young)	(0.75, 0.92)	0.17
			HHSIZGR (small household)	(0.01, 0.33)	
			OTHERO (history of other chronic conditions)	(1.27, 62.90)	
SCB52	p=0.12	p=0.12	none		
SWWV2	p=0.04	p=0.02	AGE82 (young)	(0.94, 0.99)	0.65
			AEI (high aerosol exposure)	(1.01, 1.04)	
SSNV2	p=0.17	p=0.04	AGE82 (young)	(0.95, 0.99)	0.86
			DWATER (public water supply)	(1.43, 8.87)	
			AEI (high aerosol exposure)	(1.01, 1.04)	

continued...

TABLE 125. (CONT'D)

Season/Agent	AEI significance		Significant[c] predictor variables	90% Confidence interval for the odds ratio	Goodness of fit[d]
	Initial[a]	Final[b]			
SUMMER 1983					
CKLB4X	p=0.09	p=0.13	WCONSM (drinks alot of water)	(1.16, 11.0)	0.13
CKLB4W	p=0.17	p=0.21	HHSIZGR (small household)	(0.23, 1.03)	0.75
CWWI4W	p>0.25	p>0.25	CONTACT (infrequent group contact)	(0.22, 0.68)	0.15
			HOHEDGR (little educ. HOH)	(1.00, 1.82)	
SE244	p>0.25	p>0.25	AGE82 (young)	(0.86, 0.97)	0.41
			SEX (females)	(0.85, 31.82)	
1982					
SCB25	p>0.25	p>0.25	SEX (males)	(0.02, 0.30)	0.23
			AGE82 (elderly)	(1.00, 1.05)	
SCB55	p=0.20	p=0.10	AGE82 (young)	(0.93, 0.99)	0.27
SE115	p=0.02	p=0.11	RESP (no respiratory history)	(0.03, 0.84)	0.29
			HOHOCC (farmer)	(1.21, 4.03)	
SWWV5	p=0.13	p>0.25	HOHOCC (farmer)	(1.13, 2.25)	0.78
			PNEU (pneumonia history)	(1.03, 5.34)	
1983					
SE246	p>0.25	p>0.25	AGE82 (young)	(0.90, 0.98)	0.75
			WCONSM (drinks little water)	(0.05, 0.47)	

[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p \leq .10$, then p-value indicates X^2 to remove AEI at last step in model selection, otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step in model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10 < p < 1.0$) indicates an acceptable fit.

high AEI for which no other explanation could be found to explain the aerosol exposure effect (see Section 5M). For the SPL11 episode, the cross-product terms of the significant variables (i.e., AEI x IM1, AEI x LNPPL11, IM1 x LNPPL11 and AEI x IM1 x LNPPL11) were also constructed as predictor variables to investigate interaction effects. None of the cross-product terms were significant predictor variables. Thus, concurrent polio immunization, low polio 1 antibody titer, and high aerosol exposure were independently associated with the polio 1 seroconversions in spring 1982 as three distinct risk factors. Each risk factor appears to have been responsible for some of the 13 poliovirus 1 infections observed between January and June 1982.

Analysis 2: Investigate Possible Restaurant Etiology--
The possible association of the infection episode with the frequency of eating food prepared at the two restaurants in Wilson was investigated in Analysis 2. This was done only for those response variables providing good or marginal evidence of aerosol exposure association. The predictor variables RESTA and RESTB (see Table 48) were added to the set of variables used in Analysis 1 and the same methodology used there was again employed. Variables RESTA and RESTB were obtained primarily from fecal donors (see Section 5C). Thus, for the serologic response variables, Analysis 2 was based on less than half of the observations used in Analysis 1.

The results of Analysis 2 are presented in Table 126. RESTA was a significant predictor variable for CVIR2W and especially for CKLB4X. RESTB was a significant predictor variable for CVIR2X. This analysis suggests frequent patronage of restaurant A as the probable explanation for the Klebsiella infection episode during the summer 1983 irrigation period.

Analysis 3: Exclude AEI to Investigate Alternative Explanations--
Analysis 1 was repeated, excluding AEI as a predictor varible, for those response variables in which AEI was a significant predictor variable in Analysis 1. The purpose of this analysis was to determine if other predictor variables would play the same explanatory role in the logistic regression as did AEI. Such variables could be considered alternative explanations to AEI as the possible cause of the infection episode. The results of Analysis 3 are given in Table 127.

Comparison of the results in Table 127 with the prior run for the response variable shows that no replacement variable for AEI was found in the SE090, SPL11, CVIR2W and SSNV2 episodes. For SWWV2, low income and caucasian replaced high AEI. For SE115, caucasian and large households replaced high AEI. The replacement variables can be considered alternative explanations to high AEI for SWWV2 and SE115.

Analysis 4: Investigate Route of Wastewater Exposure--
Exposure to the wastewater aerosol, direct contact with the wastewater, and spending time in the irrigation environment on the Hancock farm are three alternative routes by which infectious agents in the wastewater could be transmitted to initiate an infection episode. The relevant measures of these exposures, AEI, XDIREM (or XDIREL), and FHRSEM, were highly correlated in the study population in each exposure season (see Table P-23 of Appendix P). Thus, AEI, which was considered to be the best single measure of wastewater

TABLE 126. RESULTS OF ANALYSIS 2 - INVESTIGATE POSSIBLE RESTAURANT ETIOLOGY

Season/Agent	AEI significance		Significant[c] predictor variables	90% Confidence interval for the odds ratio	Goodness of fit[d]
	Initial[a]	Final[b]			
BASELINE					
SE090	p=0.001	p=0.001	AEI (high aerosol exposure)	(1.17, 11.28)	
SAD70	p=0.21	p>0.25	HOHEDGR (college educ. HOH)	(1.18, 5.91)	0.50
SPRING 1982					
SPL11	p=0.01	p=0.002	AEI (high aerosol exposure)	(1.04, 1.17)	0.49
			LNPPL11 (low antibody level)	(0.03, 0.68)	
			IM1 (polio immunization in Spring 82)	(1.92, 217.29)	
SUMMER 1982					
CVIR2X	p=0.18	p=0.13	RESTB (ate frequently at restaurant B)	(0.20, 0.80)	0.14
CVIR2W	p=0.22	p>0.25	HHSIZGR (large household)	(1.21, 5.89)	0.74
			RESTA (ate frequently at restaurant A)	(0.25, 0.93)	
SCB42	p>0.25	p>0.25	none		
SCB52	p=0.11	p=0.11	none		
SWWV2	p=0.02	p=0.002	AEI (high aerosol exposure)	(1.00, 1.14)	0.60
			AGE82 (young)	(0.73, 1.04)	
SSNV2	p=0.08	p=0.001	AGE82 (young)	(0.75, 0.96)	0.88
			AEI (high aerosol exposure)	(1.02, 1.14)	
			PNEU (pneumonia history)	(4.96, 3.21E6)	
			INCOME (low)	(0.02, 0.85)	
SUMMER 1983					
CKLB4X	p=0.11	p>0.25	RESTA (ate frequently at restaurant A)	(0.05, 0.44)	0.01
			WCONSM (drinks a lot of water)	(1.97, 56.24)	
			SEX (females)	(1.90, 118.60)	
			TLUBOCK (little time in Lubbock)	(0.74, 1.01)	
SE244	p>0.25	p>0.25	AGE82 (young)	(0.86, 0.97)	0.78
			DWATER (private wells)	(0.02, 0.78)	

continued...

TABLE 126 (CONT'D)

Season/Agent	AEI significance Initial[a]	 Final[b]	Significant[c] predictor variables	90% Confidence interval for the odds ratio	Goodness of fit[d]
1982					
SCB25	$p>0.25$	$p>0.25$	AGE82 (young)	(0.23, 1.19)	
SE115	$p=0.02$	$p>0.12$	SEX (males)	(0.01, 0.25)	
SWWV5	$p>0.25$	$p>0.25$	none		
1983					
SE246	$p>0.25$	$p>0.25$	AGE82 (young)	(0.86, 0.98)	0.58

a This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

b If $p \leq .10$, then p-value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

c Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

d p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 127. RESULTS OF ANALYSIS 3 - EXCLUDE AEI TO INVESTIGATE ALTERNATIVE EXPLANATIONS

Season/Agent	Significant[a] predictor variables	90% Confidence interval for the odds ratio	Goodness of fit[b]
BASELINE			
SE090	none		
SPRING 1982			
SPL11	IM1 (polio immunization in Spring 1982)	(7.45, 97.23)	0.36
	LNPPL11 (low antibody level)	(0.14, 0.48)	
SUMMER 1982			
SWWV2	AGE82 (young)	(0.93, 0.98)	0.50
	INCOME (low)	(0.38, 0.87)	
	RACE (caucasians)	(0.18, 0.74)	
CVIR2W	HHSIZGR (large household)	(1.01, 3.59)	0.72
1982			
SE115	RESP (no respiratory history)	(0.03, 0.98)	0.82
	HOHOCC (farmer)	(1.76, 9.68)	
	DWATER (public water supply)	(2.17, 31.96)	
	RACE (caucasians)	(0.31, 0.85)	
	HHSIZGR (large household)	(1.02, 3.39)	

[a] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[b] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

irrigation exposure, was the only exposure measure employed in Analysis 1. For those response variables whose regression equation in Analysis 1 contained the predictor variable AEI, Analysis 4 also was performed.

In Analysis 4, predictor variables FHRSEM and XDIREL (or XDIREM when available) were included with the previous predictor variables used in Analysis 1. The methodology of Analysis 1 again was utilized in performing the logistic regression analysis.

The results of Analysis 4 are presented in Table 128. Of the six response variables investigated during periods of irrigation, the irrigation exposure measure selected was AEI for four episodes (SPL11, SCB42, SSNV2 and SE115), XDIREL for episode CVIR2W and FHRSEM for episode SWWV2. Wastewater irrigation cannot be implicated as the source of exposure using only the logistic regression evidence. However, if wastewater irrigation was found to be a causative factor of the infection episodes investigated, the results of Analysis 4 provide evidence supporting all three exposure routes, with the aerosol exposure route having the most supporting evidence.

Evaluation of the Effect of Ignoring Multiple Infection Events on the Statistical Analysis Results

To conduct the confirmatory analysis using Fisher's exact test and the exploratory analysis using logistic regression, it was necessary to ignore multiple infection events. These analyses made the assumption that persons experiencing more than one infection event in the period of observation of an infection episode provided the same information regarding the distribution of infections as did persons experiencing a single infection event in the observation period.

The effect on each confirmatory analysis result of ignoring the multiple infection events is presented in Table 129 for each episode in which multiple infection events occurred. By noting which exposure group would have had more infection events or a higher rate of increased infection events in each such episode, the direction of the effect on the reported p-value was determined. No confirmatory analysis results would have been changed substantially. Two associations reported to be significant at $p=0.02$ (i.e., for echovirus 9 in the baseline period and for all serologically detected infections in 1982 to viruses recovered from the wastewater) were probably somewhat more significant ($p<0.02$).

The effect on each exploratory logistic regression result of ignoring multiple infection events is shown in Table 130. The AEI means of all participants with 2, 3 and 4 infection events were compared to the mean AEI of all participants with a single infection event to determine the direction of the effect of ignoring the multiple infection events. There were four infection episodes with multiple infection events in which the p-value of AEI on the final step of model construction was less than 0.10. The p-value accounting for multiple events would probably have been more significant for one of the four: SSNV2 (all serum neutralization-tested viruses in summer 1982) with $p<0.05$. Taking multiple events into account would likely have made these associations less significant: SE090 ($p>0.01$),

TABLE 128. RESULTS OF ANALYSIS 4 - INVESTIGATE ROUTE OF WASTEWATER EXPOSURE

	AEI significance			90% Confidence interval for the odds ratio	Goodness of fit[d]
Season/Agent	Initial[a]	Final[b]	Significant[c] predictor variables		
BASELINE					
SE090	p=0.02	p=0.02	AEI (high aerosol exposure)	(1.02, 1.11)	
SPRING 1982					
SPL11	p=0.01	p=0.01	IM1 (polio immunization in Spring 1982)	(7.18, 101.98)	0.93
			LNPPL11 (Low antibody level)		
			AEI (high aerosol exposure)		
SUMMER 1982					
CVIR2W	p=0.19	p>0.25	AGE82 (young)	(0.95, 0.99)	0.31
			XDIREL (extensive direct wastewater contact)	(1.20, 5.84)	
SCB42	p>0.25	p=0.01	AGE82 (young)	(0.54, 0.92)	0.35
			HOHEDGR (college educ. HOH)	(1.15, 10.78)	
			SMOKE3 (smoker)	(8.31, 1.25E7)	
			AEI (high aerosol exposure)	(1.02, 1.21)	
			RESP (respiratory history)	(0.86, 461.39)	
SWWV2	p=0.04	p>0.25	FHRSEM (frequent Hancock farm)	(1.01, 1.03)	0.92
			AGE82 (young)	(0.94, 0.99)	
SSNV2	p=0.19	p=0.05	AGE82 (young)	(0.93, 0.98)	0.27
			INCOME (low)	(0.25, 0.85)	
			SMOKE3 (smoker)	(1.21, 13.74)	
			AEI (high aerosol exposure)	(1.01, 1.04)	
			DWATER (public water supply)	(0.98, 6.61)	
			RACE (caucasians)	(0.49, 0.99)	
1982					
SE115	p=0.04	p=0.06	RESP (no respiratory history)	(0.03, 1.06)	0.33
			HOHOCC (farmer)	(1.35, 5.18)	
			DWATER (public water supply)	(1.35, 9.35)	
			AEI (high aerosol exposure)	(1.00, 1.03)	

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[a] This is the p-value of AEI at the initial step; i.e., when AEI would be the only variable in the prediction equation.

[b] If $p \leq .10$, then p-value indicates X^2 to remove AEI at last step in model selection; otherwise p-value indicates X^2 to enter AEI at last step in model selection.

[c] Predictor variables in regression model at last step of model selection; the subgroup in parentheses had the higher infection rate.

[d] p-value for Hosmer's chi-square goodness-of-fit; a large value (i.e., $.10<p<1.0$) indicates an acceptable fit.

TABLE 129. EFFECT OF MULTIPLE INFECTION EVENTS ON CONFIRMATORY ANALYSIS RESULTS

Dependent variable			Number of observations by infection status							
Agent	Season	Exposure group	0[a]	1 or more[a]	1	2	3	4	Confirmatory analysis p-value	Direction of effect on reported p-value if multiple infection events had been taken into account
VIR-X	Sum 1981	Low	15	8	6	2			>0.25	Less significant
		High	5	1	1					
VIR-W	Sum 1982	Low	73	7	5	2			0.13	Less significant (p>0.13)
		High	21	5	5					
WWI-W	Spr 1982	Low	61	8	7	1			>0.25	Less significant
		High	37	4	4					
	Sum 1982	Low	51	13	11	2			0.21	Less significant
		High	15	7	7					
CB4	1982	Low	213	13	13				>0.25	More significant
		High	68	6	5	1				
E03	Baseline	Low	156	9	8	1			>0.25	Less significant
		High	91	3	3					
E09	Baseline	Low	176	2	2				0.02	More significant (p<0.02)
		High	87	6	5	1				
E24	Baseline	Low	167	5	4	1			>0.25	Less significant
		High	94	3	3					
	1983	Low	178	4	3	1			0.02	Little effect
		High	64	6	5	1				
ROT	Baseline	Low	9	4	3	1			>0.25	Little effect
		High	10	7	6	1				
WWV	Sum 1982	Low	183	10	9	1			>0.25	Less significant
		High	52	5	5					
	1982	Low	139	41	37	4			0.02	Slightly more significant (p<0.02)
		High	34	21	18	2	1			
SNV	Baseline	Low	77	62	50	7	2	3	>0.25	Slightly more significant
		High	33	36	23	9	3	1		
	Spr 1982	Low	111	12	11	1			>0.25	Less significant
		High	52	8	8					
	Sum 1982	Low	133	17	16	1			>0.25	Slightly more significant
		High	35	5	4	1				
	1982	Low	113	58	51	6		1	0.22	Slightly more significant
		High	31	23	20	2	1			
	Sum 1983	Low	137	23	19	3	1		>0.25	More significant
		High	43	6	3	1	1	1		
	1983	Low	124	33	23	8	2		>0.25	More significant
		High	50	14	9	2	1	2		

[a] Values used in analysis.

TABLE 130. EFFECT OF MULTIPLE INFECTION EVENTS ON EXPLORATORY LOGISTIC REGRESSION ANALYSIS RESULTS

Dependent variable by season	Mean AEI (number) of observations by infection status						Exploratory logistic regression final step p-value	Direction of effect on reported AEI p-value if multiple infection events had been taken into account
	Recoded observations		Actual observations					
	0	1	1	2	3	4		
Baseline								
SED90	3.84(263)	13.08(8)	14.32(7)	4.53(1)			0.01	Less significant (p>0.01)
Summer 1982								
CVIR2W	6.44(94)	17.10(12)	20.17(10)	1.75(2)			0.07	Less significant (p>0.07)
SWWV2	4.53(235)	16.93(15)	17.98(14)	2.25(1)			0.02	Less significant (p>0.02)
SSNV2	5.17(168)	11.98(22)	11.75(20)	14.20(2)			0.05	More significant (p<0.05)
1982								
SC845	5.53(281)	7.01(19)	3.25(18)	74.71(1)			>0.25	Much more significant (p<0.25?)
SWWV5	5.05(173)	9.16(62)	7.98(55)	17.04(6)	26.51(1)		>0.25	More significant (p<0.25?)
SSNV5	5.48(144)	7.80(81)	7.04(71)	12.95(8)	26.51(1)	1.79(1)	>0.25	More significant (p<0.25?)
1983								
SE246	6.82(242)	3.75(10)	3.92(8)	3.09(2)			>0.25	Little effect
SSNV6	6.01(174)	7.64(47)	9.97(32)	2.35(10)	2.04(3)	5.08(2)	>0.25	Less significant (p>0.25)

CVIR2W ($p>0.07$) and SWWV2 ($p>0.02$). Because there was a small proportion of multiple infection events in each of these episodes, the magnitude of the change in p-value is unlikely to have been large. For only three of the episodes (SWWV5, SSNV5 and SSNV6) did enough multiple infection events occur to have allowed a valid exploratory analysis of their effect using a weighted least squares approach.

M. EVIDENCE OF ASSOCIATION OF SPECIFIC INFECTION EPISODES WITH WASTEWATER AEROSOL EXPOSURE

The LISS has employed four methods of inference to investigate the possible association of infections with wastewater aerosol exposure in the episodes of infection which were observed in the study population. These inferential methods were: 1) risk ratio (RR) scoring (see Section 5K), 2) the incidence density ratio (IDR) of high-to-intermediate and high-to-low exposure levels for serologic infection episodes (see Section 5I), 3) confirmatory statistical analysis (CA) (see Section 5L), and 4) exploratory logistic regression (ELR) statistical analysis (see Section 5L). Five scores were assigned to every infection episode based on the results obtained by each of the four methods.

The RR score is a classification of an infection episode by comparison of the infection incidence rates in the low ($AEI<3$) and high ($AEI\geq 3$) exposure groups and in the low ($AEI<1$), intermediate ($1\leq AEI\leq 5$) and high ($AEI>5$) exposure levels. The high and low exposure groups and levels are treated in a symmetric manner in assigning the RR score.

Two incidence density (ID) ratios for the exposure levels (i.e., ID_{Hi}/ID_{Int} and ID_{Hi}/ID_{Lo}) were calculated for each serologic infection episode. The 90% and 95% confidence intervals (CI) were constructed for each IDR for which two or more infection events were expected in both of the compared levels to determine if the intervals included the value 1.00. Two IDR scores which are assigned on this basis also evaluate the possible association of infections with aerosol exposure.

The confirmatory statistical analysis used Fisher's exact test to test the hypothesis that the infection rates within the low and high exposure groups were equal for each infection episode, against the one-sided alternative that the high exposure group had a larger infection rate. The confirmatory analysis score is assigned based on the p-value of this test.

The exploratory statistical analysis used the stepwise logistic regression method to investigate whether the presence of infection was associated with the degree of exposure measured by the aerosol exposure index (AEI), controlling for the effect of significant monitored covariates. An analysis was performed for each infection episode for which a higher infection rate was observed in the high exposure group than in the low exposure group and in the high exposure level than in the intermediate and low exposure levels. A multiple linear logistic regression model was formed in a stepwise fashion, with one predictor variable with a chi-square p-value below 0.10 entering the model or one predictor variable with chi-square p-value above 0.15 removed from the model at each step. The exploratory analysis score is based on the p-value of chi-square to enter or remove the AEI predictor variable at the last step of the model selection process.

A summary containing the scores from each of these inferential methods is presented for each control infection episode in Table 131 and for each exposure infection episode in Table 132. The actual p-value of the CA result is given in parentheses after the score when $p \leq 0.15$. The actual p-values of the AEI predictor variable are given in parentheses for the ELR results both initially and at the final step, whenever the respective $p \leq 0.25$. The initial step p-value suggests the apparent degree of association of infections with AEI, uncontrolled for other factors. In contrast, the final step p-value indicates the degree of association of infections with AEI, controlling for the other significant predictor variables (which are also in the model at the last step).

Tables 131 and 132 indicate that, as expected, a number of the statistically significant associations found by the methods employed in certain infection episodes were not supported by the results from the other inferential methods. It is important to identify the infection episodes for which there is strong and consistent evidence of association among the inferential methods, since these infection episodes warrant additional scrutiny.

The four inferential methods complement each other to provide a balanced assessment of the association of infection events with wastewater aerosol exposure in a specific infection episode. Since each method also has its deficiencies, all four methods are needed to achieve a proper interpretation about the strength of the association.

The RR score, CA and ELR all ignore multiple infection events in the episode, in that they place each participant with one or more infection events in the same group, the ''infected donors.'' In contrast, the IDR takes multiple infection events properly into account. However, the IDR confidence intervals will be inaccurate, and thus are not used, when the number of observed infection events is small.

The confirmatory analysis is conducted with known power to permit assessment of the frequency of positive associations found. However, CA lacks the ability to investigate association with degree of exposure. Thus, participants with very high (e.g., $AEI > 50$) and intermediate ($3 \leq AEI < 5$) aerosol exposure are treated as having the same amount of exposure. Effects occurring only in highly exposed subjects are unlikely to be detected by CA.

The exploratory logistic regression analysis investigates association with the degree of aerosol exposure while it controls for the effects of significant covariates. However, various ELR models often have nearly equivalent goodness of fit. While the model presented at the final step of the stepwise ELR procedure usually has acceptable goodness of fit, it may not be the best fitting model. Hence, the selection sequence will sometimes fail to choose AEI as a significant variable when aerosol exposure may actually be important, especially when AEI is highly correlated with another variable. Alternative explanations to AEI must be investigated

TABLE 131. SUMMARY OF FINDINGS FOR CONTROL INFECTION EPISODES: EVIDENCE REGARDING SPURIOUS ASSOCIATION OF INFECTIONS WITH WASTEWATER AEROSOL EXPOSURE

''Control'' infection episode		Jointly indep. episode group[a]	No. inf.[b]	Infection risk ratios[c]		Risk ratio score[d]	Scores of serologic incidence density ratio of exp levels[e]		Statistical analysis results			Strength and consistency of apparent association of infections with exposure[h]
Agent Obs period	Depend. var.			Exp group RR	Exp level RR		Hi/Int	Hi/Lo	Confirm. analysis score[f] (p-value)	Exploratory: AEI significance[g] score (p-value) Initial	Final	
Clinical (C)												
VIR (Viruses, excluding adeno and immunization polio)												
8 (Sum 80)	CVIR8	C	12	1.8	–	0	nd[i]	nd	–		–	
9 (Sum 81)	CVIR9	C	9	0.5	–	—	nd	nd	–		(–)	
Serologic (S)												
AD3 (Adeno 3)												
Baseline	SAD30	C	13	0.5	0	0	–	–	–		(–)	
AD5 (Adeno 5)												
Baseline	SAD50	C	7	1.4	0	0	–	–	–		(–)	
AD7 (Adeno 7)												
Baseline	SAD70	C	6	3.8	4.2	++	0	0	0 (0.11)	(0.12)	0 (0.23)	
CB2 (Coxsackie B2)												
Baseline	SCB20	C	14	0.7	0.5	0	–	–	–		(–)	
CB4 (Coxsackie B4)												
Baseline	SCB40	C	16	1.1	0	0	–	–	–		(–)	
CB5 (Coxsackie B5)												
Baseline	SCB50	C	11	2.4	4.2	+	–	0	0 (0.12)		–	
E01 (Echo 1)												
Baseline	SE010	C	7	1.5	0	0	–	–	–		(–)	
E03 (Echo 3)												
Baseline	SE030	C	12	0.6	0.2	–	–	–	–		(–)	
E09 (Echo 9)												
Baseline	SE090	C	8	5.6	1.9	+	–	0	++ (0.02)	(0.03)	++ (0.01)	Good
E11 (Echo 11)												
Baseline	SE110	C	17	1.4	0.6	0	–	–	–		(–)	
E20 (Echo 20)												
Baseline	SE200	C	5	0.4	0	0	–	–	–		(–)	
E24 (Echo 24)												
Baseline	SE240	C	8	1.0	0.9	0	–	–	–		(–)	
PL1 (Polio 1)												
Baseline	SPL10		69	1.0	1.7	0	0	0			(–)	
67 Salk immun adults:		C	49	1.0	1.1	0	0	0	–			
34 Sabin immun children:		C	17	0.8	1.8	0	0	0	–			
PL2 (Polio 2)												
Baseline	SPL20		72	1.1	1.3	0	0	0			(–)	
67 Salk immun adults:		C	51	1.0	1.0	0	0	0	–			
33 Sabin immun children:		C	19	1.1	0.9	0	–	–	–			

continued...

TABLE 131. (CONT'D)

''Control'' infection episode Agent Obs period	Depend. var.	Jointly indep. episode group[a]	No. inf.[b]	Infection risk ratios[c] Exp group RR	Exp level RR	Risk ratio score[d]	Scores of serologic incidence density ratio of exp levels[e] Hi/Int	Hi/Lo	Statistical analysis results Confirm. analysis score[f] (p-value)	Exploratory: AEI significance[g] score (p-value) Initial	Final	Strength and consistency of apparent association of infections with exposure[h]
PL3 (Polio 3)												
Baseline	SPL30		71	1.3	2.0	0	0	+			-	
67 Salk immun adults:		C	56	1.1	1.2	0	0	0	-			
32 Sabin immun children:		C	15	1.2	2.1	0	0	0	-			
RE1 (Reo 1)												
Baseline	SRE10	C	35	0.4	0.4	-	-	-	—		(-)	
RE2 (Reo 2)												
Baseline	SRE20	C	37	0.8	1.5	0	-	0	-	(0.12)	-	
ROT (Rotavirus)												
Baseline	SROTO	C	11	1.3	Large	+	-	0	-		-	
INA (Influenza A)												
0 (80-81)	SINA0	C	19	0.9	2.7	0	-	0	-		-	
1 (81-82)	SINA1	C	6	1.1	0	0	-	-	-		(-)	
3 (82-83)	SINA3	C	35	0.7	0.7	0	-	-	-		(-)	
POR (Sporadic serum neutralization viruses)												
Baseline	SPORO	C	8	1.0	0.7	0	nd	nd	-		(-)	
SNV (All serum neutralization viruses)												
Baseline	SSNVO	F	98	1.1	0.6	0	-	-	-		(-)	

a Classification criteria for the jointly independent groups of control infection episodes are given in Table 15.

b Based on all observed individuals for whom an AEI exposure estimate was available.

c $RR=IR_{Hi}/IR_{Lo}$.

d From Table 101.

e From Tables 88 and P-47 in Appendix P. The score criterion for the incidence density ratio (IDR) is based on its confidence interval (CI):

- $IDR \leq 1.0$ + 90% CI does not include 1.0
0 $IDR > 1.0$, but 90% CI includes 1.0 ++ 95% CI does not include 1.0

f From Tables 110 to 113. The confirmatory analysis score criterion is based on the p-value for the one-tailed Fisher's exact test:

— $p \geq 0.95$ + $0.05 < p \leq 0.10$
- $0.95 > p > 0.15$ ++ $0.01 < p \leq 0.05$
0 $0.10 < p \leq 0.15$ +++ $p \leq 0.01$

g From Tables 118 to 125. The exploratory analysis score criterion is based on the p-value of chi-square to enter or to remove for the AEI predictor variable at the last step of the logistic regression model selection:

(-) Exploratory analysis not performed because group or level $RR \leq 1.0$ + $0.05 < p \leq 0.10$
++ $0.01 < p \leq 0.05$
- $p > 0.25$ +++ $p \leq 0.01$
0 $0.10 < p \leq 0.25$

The p-value of chi-square to enter AEI at the initial step is also presented when $p \leq 0.25$.

h Criteria for good and marginal strength and consistency of association are given in Table 19.

i nd - analysis not done.

TABLE 132. SUMMARY OF FINDINGS FOR EXPOSURE INFECTION EPISODES: EVIDENCE REGARDING ASSOCIATION OF INFECTIONS WITH WASTEWATER AEROSOL EXPOSURE

''Exposure'' infection episode		Jointly indep. episode group[a]	No. inf.[b]	Infection risk ratios[c]		Risk ratio score[d]	Scores of serologic incidence density ratio of exp levels[e]		Statistical analysis results			Strength and consistency of apparent association of infections with exposure[h]
Agent Obs period	Depend. var.			Exp group RR	Exp level RR		Hi/Int	Hi/Lo	Confirm. analysis score[f] (p-value)	Exploratory: AEI significance[g] score (p-value) Initial	Final	
Clinical (C)												
KLB (Klebsiella)												
2 (Sum 82)	CKLB2X	A	5	1.9	0	0	nd[i]	nd	–		(–)	
	CKLB2W		13	1.3	0	0	nd	nd	–		(–)	
4 (Sum 63)	CKLB4X	A	8	4.5	5.6	++	nd	nd	++ (0.03)	(0.09)	0 (0.13)	Good
	CKLB4W		12	3.6	3.4	++	nd	nd	++ (0.02)	(0.17)	0 (0.21)	
OOB (Other opportunistic bacteria)												
3 (Spr 83)	COOB3	A	5	1.9	1.4	0	nd	nd	–		–	
PBW (Prominent bacteria in wastewater)												
1 (Spr 82)	CPBW1W	A	3	0.8	1.0	0	nd	nd	–		(–)	
2 (Sum 82)	CPBW2X	A	3	1.4	Large	0	nd	nd	–		–	
	CPBW2W		4	2.7	Large	+	nd	nd	–		–	
4 (Sum 83)	CPBW4W	A	9	1.3	1.4	0	nd	nd	–		(–)	
VIR (Viruses, excluding adeno and immunization polio)												
1 (Spr 82)	CVIR1X	A	9	0.9	0.8	0	nd	nd	–		(–)	
	CVIR1W		15	0.7	1.1	0	nd	nd	–		(–)	
2 (Sum 82)	CVIR2X	A	11	2.5	3.1	++	nd	nd	+ (0.10)	(0.16)	0 (0.16)	
	CVIR2W		12	2.2	3.1	+	nd	nd	0 (0.13)	(0.18)	+ (0.07)	Marginal
4 (Sum 83)	CVIR4W	A	5	0.7	Large	0	nd	nd	–		(–)	
WWI (Agents isolated from wastewater)												
1 (Spr 82)	CWWI1X	D	7	1.2	2.0	0	nd	nd	–		–	
	CWWI1W		12	0.8	1.2	0	nd	nd	–		(–)	
2 (Sum 82)	CWWI2X	D	12	1.6	2.7	0	nd	nd	–		–	
	CWWI2W		20	1.6	1.4	0	nd	nd	–		(–)	
3 (Spr 83)	CWWI3	D	4	1.3	1.4	0	nd	nd	–		–	
4 (Sum 83)	CWWI4X[j]	D	8	4.8	5.8	++	nd	nd	++ (0.02)	(0.11)	0 (0.16)	Marginal
	CWWI4W		22	2.2	2.4	+	nd	nd	++ (0.03)		–	
Serologic (S)												
AD3 (Adeno 3)												
5 (1982)	SAD35	B	7	0.5	0	0	–	–	–		(–)	
AD5 (Adeno 5)												
5 (1982)	SAD55	B	8	0	0	0	–	–	–		(–)	
CB2 (Coxsackie B2)												
5 (1982)	SCB25	B	9	3.7	Large	+	0	0	++ (0.05)		–	

continued...

TABLE 132. (CONT'D)

''Exposure'' infection episode		Jointly indep. episode group[a]	No. inf.[b]	Infection risk ratios[c]		Risk ratio score[d]	Scores of serologic incidence density ratio of exp levels[e]		Statistical analysis results			Strength and consistency of apparent association of infections with exposure[h]
Agent Obs period	Depend. var.			Exp group RR	Exp level RR		Hi/Int	Hi/Lo	Confirm. analysis score[f] (p-value)	Exploratory: AEI significance[g] score (p-value) Initial	Final	
CB4 (Coxsackie B4)												
2 (Sum 82)	SCB42	A	5	2.3	5.2	+	0	0	–		0 (0.16)	
5 (1982)	SCB45	B	18	1.5	1.4	0	++	0	–		–	
CB5 (Coxsackie B5)												
1 (Spr 82)	SCB51	A	4	1.8	0	0	–	–	–		(–)	
2 (Sum 82)	SCB52	A	4	3.3	Large	+	0	0	–	(0.12)	0 (0.12)	
5 (1982)	SCB55	B	8	1.8	7.5	0	0	0	–	(0.20)	0 (0.10)	
4 (Sum 83)	SCB54	A	8	0	0	0	–	–	–		(–)	
6 (1983)	SCB56	B	9	0.3	0.9	0	–	–	–		(–)	
E03 (Echo 3)												
5 (1982)	SE035	B	3	0.4	0.6	–	0	–	–		(–)	
4 (Sum 83)	SE034	A	11	1.3	4.0	0	–	0	–		–	
6 (1983)	SE036	B	18	1.7	2.4	+	0	0	–		–	
E11 (Echo 11)												
1 (Spr 82)	SE111	A	4	1.9	0	0	–	–	–		(–)	
2 (Sum 82)	SE112	A	7	1.4	2.9	0	0	0	–	(0.11)	0 (0.11)	
5 (1982)	SE115	B	19	2.2	3.0	+	++	++	+ (0.07)	(0.02)	0 (0.11)	Good
4 (Sum 83)	SE114	A	6	3.3	1.9	0	–	0	0 (0.14)		–	
6 (1983)	SE116	B	10	2.6	1.2	+	0	0	0 (0.11)		–	
E19 (Echo 19)												
5 (1982)	SE195	B	3	6.0	Large	+	0	0	–		–	
E20 (Echo 20)												
4 (Sum 83)	SE204	A	6	1.7	1.8	0	0	0	–		–	
6 (1983)	SE206	B	9	0.7	1.7	0	0	0	–		(–)	
E24 (Echo 24)												
5 (1982)	SE245	B	7	0.5	0.6	–	0	–	–		(–)	
4 (Sum 83)	SE244	A	7	4.4	Large	+	0	0	+ (0.05)		–	
6 (1983)	SE246	B	10	3.9	5.1	+	0	0	++ (0.03)		–	
PL1 (Polio 1)												
1 (Spr 82)	SPL11		13	5.2	Large	++	0	++	++ (0.02)	(0.01)	++ (0.01)	Good
61 polio immunized:		A	8	5.9	Large	+	0	0	++ (0.04)			
186 not immunized:		A	5	2.9	Large	+	0	0	–			

continued...

TABLE 132. (CONT'D)

"Exposure" infection episode Agent Obs period	Depend. var.	Jointly indep. episode group[a]	No. inf.[b]	Infection risk ratios[c] Exp group RR	 Exp level RR	Risk ratio score[d]	Scores of serologic incidence density ratio of exp levels[e] Hi/Int	 Hi/Lo	Statistical analysis results Confirm. analysis score[f] (p-value)	Exploratory: AEI significance[g] score (p-value) Initial	 Final	Strength and consistency of apparent association of infections with exposure[h]
PL2 (Polio 2)												
1 (Spr 82)	SPL21		9	5.7	2.8	++	0	0	+ (0.07)	(0.11)	-	
61 polio immunized:		A	7	5.1	2.0	+	0	0	+ (0.08)			
PL3 (Polio 3)												
1 (Spr 82)	SPL31		7	1.4	1.4	0	-	0	-			
61 polio immunized:		A	7	1.1	0.7	0	-	-	-			
RE1 (Reo 1)												
1 (Spr 82)	SRE11	A	16	0.8	0.7	0	-	-	-		(-)	
RE2 (Reo 2)												
1 (Spr 82)	SRE21	A	13	0.5	0	0	-	-	-		(-)	
ROT (Rotavirus)												
1 (Spr 82)	SROT1	A	3	2.0	Large	0	0	0	-		-	
2 (Sum 82)	SROT2	A	4	6.0	2.1	+	0	0	0 (0.10)		-	
5 (1982)	SROT5	B	7	2.1	2.1	+	0	0	-		-	
3 (Spr 83)	SROT3	A	3	1.6	Large	0	0	0	-		0 (0.21)	
4 (Sum 83)	SROT4	A	6	1.1	Large	0	0	0	-		-	
6 (1983)	SROT6	B	9	1.0	1.0	0	0	0	-		(-)	
LEG (Legionella pneumophila 1)												
1981-83	SLEG7	B	6	1.1	0.7	0	0	0	-		(-)	
POR (Sporadic serum neutralization viruses)												
1 (Spr 82)	SPOR1	A	13	0.9	1.2	0	0	0	-		(-)	
2 (Sum 82)	SPOR2	A	9	0.5	2.5	0	-	0	-		(-)	
5 (1982)	SPOR5	B	5	0	0	0	-	-	-		(-)	
6 (1983)	SPOR6	B	10	0.8	0.8	0	-	-	-		(-)	
WWV (Viruses isolated from wastewater)												
1 (Spr 82)	SWWV1	D	12	1.0	0	0	-	-	-		(-)	
2 (Sum 82)	SWWV2	D	15	1.7	4.8	+	++	++	-	(0.04)	++ (0.02)	Good
5 (1982)	SWWV5	E	61	1.7	1.8	+	++	++	++ (0.02)	(0.13)	-	Good
6 (1983)	SWWV6	E	11	0.6	0.9	0	-	-	-		(-)	

continued...

TABLE 132. (CONT'D)

''Exposure'' infection episode: Agent Obs period	''Exposure'' infection episode: Depend. var.	Jointly indep. episode group[a]	No. inf.[b]	Infection risk ratios[c]: Exp group RR	Infection risk ratios[c]: Exp level RR	Risk ratio score[d]	Scores of serologic incidence density ratio of exp levels[e]: Hi/Int	Scores of serologic incidence density ratio of exp levels[e]: Hi/Lo	Statistical analysis results: Confirm. analysis score[f] (p-value)	Statistical analysis results: Exploratory: AEI significance[g] score (p-value): Initial	Statistical analysis results: Exploratory: AEI significance[g] score (p-value): Final	Strength and consistency of apparent association of infections with exposure[h]
SNV (All serum neutralization viruses)												
1 (Spr 82)	SSNV1		20	1.3	0.8	0	-	-	-		(-)	
2 (Sum 82)	SSNV2		22	1.1	3.4	0	0	++	-	(0.17)	++ (0.04)	Marginal
5 (1982)	SSNV5		81	1.3	1.5	0	++	++	0 (0.15)		-	
3 (Spr 83)	SSNV3	D	12	0.4	0.5	-	-	-	-		(-)	
4 (Sum 83)	SSNV4	D	29	0.9	1.6	0	-	+	-		(-)	
6 (1983)	SSNV6		47	1.0	1.5	0	0	+	-		-	

[a] Classification criteria for the jointly independent groups of exposure infection episodes are given in Table 15.

[b] Based on all observed individuals for whom an AEI exposure estimate was available.

[c] RR=IR_{Hi}/IR_{Lo}.

[d] From Table 100.

[e] From Tables 88 and P-47 in Appendix P. The score criterion for the incidence density ratio (IDR) is based on its confidence interval (CI):

- IDR≤1.0
0 IDR>1.0, but 90% CI includes 1.0
+ 90% CI does not include 1.0
++ 95% CI does not include 1.0

[f] From Tables 110 to 113. The confirmatory analysis score criterion is based on the p-value for the one-tailed Fisher's exact test:

— $p \geq 0.95$
- $0.95 > p > 0.15$
0 $0.10 < p \leq 0.15$
+ $0.05 < p \leq 0.10$
++ $0.01 < p \leq 0.05$
+++ $P \leq 0.01$

[g] From Tables 118 to 125. The exploratory analysis score criterion is based on the p-value of chi-square to enter or to remove for the AEI predictor variable at the last step of the logistic regression model selection:

(-) Exploratory analysis not performed because group or level RR ≤1.0
- $p > 0.25$
0 $0.10 < p \leq 0.25$
+ $0.05 < p \leq 0.10$
++ $0.01 < p \leq 0.05$
+++ $p \leq 0.01$

The p-value of chi-square to enter AEI at the initial step is also presented when $p \leq 0.25$.

[h] Criteria for good and marginal strength and consistency of association are given in Table 19.

[i] nd - analysis not done.

[j] All CWWI4X infections are Klebsiella (i.e., CWWI4X is virtually the same as CKLB4X).

when it is selected in the final model, since spurious variables can enter stepwise regression models.

The risk ratio score provides a good overview because it examines the infection incidence rates of both the exposure groups and the exposure levels simultaneously. However, the RR score cannot assess the statistical significance of the apparent associations which it identifies.

The strength of the association of infections and exposure in an infection episode was determined based on the most statistically significant result from the CA, ELR and IDR methods. Consistency in support of the association among the other inferential methods (CA, ELR, IDR and RR score) was also required. The precise criteria which were employed to classify the strength and consistency of the evidence of association in a specific infection episode as ''good'' or ''marginal'' based on the four inferential methods were given in Table 19.

The infection episodes classified as having good or marginal evidence of a strong and consistent association are identified in the last column of Tables 131 and 132. The six infection episodes for which good evidence of a strong and consistent association was found are:

''Good'' evidence:

- SE090 (echovirus 9 seroconversions in baseline)
- CKLB4X (Klebsiella infections in summer 1983)
- SE115 (echovirus 11 seroconversions in 1982)
- SPL11 (poliovirus 1 seroconversions in spring 1982)
- SWWV2 (seroconversions to viruses isolated from wastewater in summer 1982)
- SWWV5 (seroconversions to viruses isolated from wastewater in 1982)

The infection episodes for which marginal evidence of a weaker or less consistent association was present are:

''Marginal'' evidence:

- CVIR2W (clinical viral infections excluding adeno and immunization polio in summer 1982)
- CWWI4X (clinical infections to agents isolated from wastewater in summer 1983) (Note: all eight CWWI4X infections were Klebsiella infections)
- SSNV2 (all seroconversions to serum neutralization-tested viruses in summer 1982)

It should be noted that SE090 is a control infection episode. This obviously spurious asociation with aerosol exposure demonstrates the necessity of investigating whether the apparent associations identified for the episodes listed above may also have alternative explanations.

The infection events of some listed infection episodes are subsets of the infection events of other listed episodes. For example, SE115 overlaps SWWV2; both are partial subsets of SWWV2, which is itself a subset of SSNV2.

The eight CWWI4X infection events are the eight Klebsiella infection events which comprise CKLB4X. Since Klebsiella was the agent of the CWWI4X infection episode and since CKLB4X provided better evidence of association, CWWI4X will be dropped from further scrutiny in deference to CKLB4X.

The LISS obtained additional pertinent information which was not employed in the inferential methods used to compile the list of eight infection episodes with good or marginal evidence of association with aerosol exposure. Enteroviruses recovered from regular wastewater samples were identified (see Tables P-5 in Appendix P, 25-27 and 39). Thus, whether the specific agent(s) of the infection episode were recovered from the wastewater during the irrigation period can be ascertained. A relative aerosol exposure measure (RAEM) was calculated for each microorganism group monitored in the aerosol sampling (see Table 42). Comparison of the period of occurrence of the infection episode to the RAEM rank of the agent's microorganism group in that season can determine whether the episode occurred in the season of highest exposure to the agent via wastewater aerosols. Alternative sources of exposure were also investigated. Contaminated drinking water was evaluated for the subset of under 20 households whose drinking water wells were being monitored at the time of the infection episode (see Table 46). The definition of a contaminated well and the procedures used to determine association with infected donors were given in Section 5C.

A retrospective survey of routine fecal and requested throat swab donors was conducted to determine the frequency with which they had eaten food prepared at each of the restaurants in Wilson. Eating frequently at restaurant A was found to be highly associated with aerosol exposure among fecal donors (see Table 109). A special ELR analysis (Analysis 2) was performed to evaluate the restaurant etiology as an alternative explanation to wastewater aerosol exposure (see Table 126). Eating at the restaurants was evaluated both as an alternative and as an additional explanation. Another ELR analysis (Analysis 3) was performed to investigate alternative explanations besides the restaurants. AEI was excluded from the eligible predictor variables for infection episodes in which it had been significant to determine if another variable would enter the model in its place.

A summary of the evidence from all of the additional data sources described above is presented in Table 133 for each of the eight infection episodes with good or marginal evidence of wastewater aerosol exposure association. A review of this evidence regarding an apparently associated episode may discredit the association by identifying a more plausible alternative explanation. Any episodes surviving this winnowing process are more likely to be causally related to wastewater aerosol exposure.

For several of the episodes in Table 133, a more plausible alternative explanation was identified. For CKLB4X, frequently eating food prepared by restaurant A was identified by ELR Analysis 2 as the most significant predictor variable of the Klebsiella infections. In addition, the episode occurred in summer 1983, which was only the third highest season of aerosol exposure to fecal coliforms. Thus, eating food prepared by restaurant A is considered the more likely explanation for this Klebsiella infection episode. For the spuriously associated episode SE090, there was evidence

TABLE 133. SUMMARY OF EVIDENCE FOR INFECTION EPISODES SHOWING STRONG ASSOCIATION OF INFECTIONS WITH WASTEWATER AEROSOL EXPOSURE

Inf. episodes with strong and consistent evidence of aerosol exposure seen			Evidence of aerosol exp. association									
						Stat anal						
Agent Obs period	Depend. var.	No. inf.	RR score	IDR scores H/I	IDR scores H/L	CA p	ELR final p	Evidence of AEI seen[a]	Recovery of agent in irrig. wastewater[b]	Rank of irrig. period by RAEM aerosol dose[c]	Assoc. with contam. drink. water?[d] p	Alternative explanations
GOOD EVIDENCE OF ASSOCIATION												
Control Situation												
ED9 (Echo 9)												
Baseline	SED90	8	+	–	0	0.02	0.01	Good	No	na[e]	na	None[f] Within family spread[g]
Exposure Situations												
KLB (Klebsiella)												
4 (Sum 83)	CKLB4X	8	++	ni[h]	ni	0.03	0.13	Good	Presumed	3	No	Eating frequently at restaurant A[f]
E11 (Echo 11)												
5 (1982)	SE115	19	+	++	++	0.07	0.11	Good	Yes: 3-8-82 3-16-82 3-22-82 8-2-82 8-4-82	Higher year	Maybe 0.21	Caucasians and large households[f]
PL1 (Polio 1)												
1 (Spr 82)	SPL11	13	++	0	++	0.02	0.01	Good	Yes: 3-8-82 3-22-82 4-19-82	3	No	None[f]
61 polio immunized:		8	+	0	0	0.04						
186 not immunized:		5	+	0	0	0.21						
WWV (Viruses isolated from wastewater)												
2 (Sum 82)	SWWV2	15	+	++	++	0.24	0.02	Good	By def.	1	Maybe 0.23 [HH125]	Low income and caucasians[f]
5 (1982)	SWWV5	61	+	++	++	0.02	>0.25	Good	By def.	Higher year	No	Farmers and pneumonia history[i]

continued...

TABLE 133. (CONT'D)

Inf. episodes with strong and consistent evidence of aerosol exposure seen Agent Obs period	Depend. var.	No. inf.	RR score	Evidence of aerosol exp. association IDR scores H/I	 H/L	Stat anal CA p	 ELR final p	Evidence of AEI ascn[a]	Recovery of agent in irrig. waste-water[b]	Rank of irrig. period by RAEM aerosol dose[c]	Assoc. with contam. drink. water?[d] p	Alternative explanations
MARGINAL EVIDENCE OF ASSOCIATION												
Exposure Situations												
VIR (Viruses, excluding adeno and immunization polio)												
2 (Sum 82)	CVIR2W	12	+	ni	ni	0.13	0.07	Marginal	Yes. Agents of 5-10 inf. recovered	1	No	Eating frequently at at restaurant A[f]
SNV (All serum neutralization viruses)												
2 (Sum 82)	SSNV2	22	0	0	++	>0.25	0.04	Marginal	Some recovered	1	Maybe 0.22 [HH125]	None[f]

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a From Tables 131 and 132.

b From Tables 22, 23, P-5 in Appendix P, 25-27 and 39.

c From Table 42. For single irrigation periods, rank of 1 is if period of observation covers irrigation season of highest aerosol dose, rank of 4 if observation period covers irrigation season of lowest aerosol dose.

d From Table 46.

e na - not applicable.

f From exploratory logistic regression (Tables 126 or 127 vs. Table 125)

g Three infected donors in same household.

h ni - not investigated.

i From Table 125.

of within household spread of the echo 9 infections in ''high exposure'' household 451: the youngest child had two seroconversions and his two next older siblings had one seroconversion each to echo 9 among the six family members observed during the baseline period.

Episode CVIR2W had only marginal evidence of aerosol exposure association. CVIR2W occurred in summer 1982, the season of maximum aerosol exposure to enteroviruses. For five of the ten infections to coxsackieviruses and echoviruses in CVIR2W, the specific agent was also recovered and identified from the wastewater sprayed during the summer 1982 irrigation. However, eating frequently at restaurant A was identified by ELR as an alternative explanation to AEI. The statistical evidence does not permit an inference whether eating at restaurant A or aerosol exposure is a more probable explanation for the CVIR2W episode.

Episodes SE115, SWWV2 and SWWV5 displayed good evidence of aerosol exposure association, but alternative explanations were identified by ELR for each of these episodes. Echovirus 11 was recovered from the wastewater on five occasions during the 1982 irrigation periods. All SWWV2 and SWWV5 agents were also recovered, because this was the definition of the WWV episodes. All three episodes also occurred in the year or season of highest enterovirus aerosol exposure. However, two SWWV2 infected donors in a very high exposure household on the Hancock farm obtained their drinking water from a well which was heavily contaminated in June 1982. The SE115 infections also might be associated with contaminated drinking water. The infection rate among donors who drank contaminated water was much higher, both for SE115 and SWWV2, but the p-values for the association were only 0.21 and 0.23, respectively, possibly due to the small sample sizes. Exploratory logistic regression identified caucasians and large households as a better fitting alternative explanation to high aerosol exposure for SE115. Low income households and caucasians were selected by ELR as a poorer fitting alternative explanation to high AEI for SWWV2. ELR found that SWWV5 infections were not related to degree of aerosol exposure. Instead, ELR selected farmers and a history of pneumonia as predictor variables for SWWV5. The evidence of episodes SE115, SWWV2 and SWWV5 is inconclusive regarding whether aerosol exposure or the identified alternative explanation(s) were the actual risk factors in these episodes.

There is only marginal evidence from the four inferential methods that episode SSNV2 was associated with wastewater aerosol exposure. The episode occurred in summer 1982, the season of highest aerosol exposure to enteroviruses. Based on very fragmentary contaminated drinking water data (including the two donors from the very high exposure household on the Hancock farm in the SWWV2 episode above), there is an indication, albeit nonsignificant at p=0.22, that episode SSNV2 might be associated with contaminated drinking water. However, no alternative explanations to AEI were identified by ELR for SSNV2. The available evidence indicates the association of SSNV2 with aerosol exposure is better than marginal, but still inconclusive because the alternative explanation of contaminated drinking water is quite plausible.

There is strong evidence that the poliovirus 1 seroconversions in spring 1982 were associated with wastewater aerosol exposure. Furthermore, SPL11 is the only infection episode in which all four inferential methods provided evidence of a significant association. The Cochran-Mantel-Haenszel confirmatory analysis showed a significant association ($p=0.02$) of polio 1 seroconversions between January and June 1982 with the high aerosol exposure group in the spring 1982 irrigation, when controlling for the effects of polio immunizations during this time period. The groups were balanced regarding previous polio 1 titers. ELR selected polio immunization in spring 1982, low prior antibody level, and a high degree of aerosol exposure as strong predictor variables for SPL11 seroconversions in a well-fitting logistic model. Each variable may be considered a distinct risk factor for polio 1 seroconversions since each made a strong contribution to the ELR model. No alternative explanations to high AEI were identified by ELR. Poliovirus 1 was recovered three times from the pipeline wastewater sprayed in spring 1982. Therefore, the poliovirus 1 seroconversions in spring 1982 provide substantial evidence of a causal association with wastewater aerosol exposure.

It is noteworthy that spring 1982 is estimated to be one of the irrigation periods in which the more highly exposed LISS participants received a relatively low cumulative dose of enteroviruses from wastewater aerosol exposure (see Table 42). Because poliovirus serology was not performed after June 1982, any poliovirus seroconversions occurring thereafter were not observed by the LISS. Summer 1982 appears to have been the season of highest poliovirus aerosol exposure (see Tables 39 and 42), with summer 1983 a distant second. Therefore, in order to fully assess the relationship between infections and wastewater aerosol exposure, it would be necessary to perform the poliovirus serology through October 1983 and to analyze any observed poliovirus infection episodes.

SECTION 6

DISCUSSION

A. PRIOR WASTEWATER AEROSOL HEALTH EFFECT STUDIES

Measuring the effect of wastewater aerosol exposure on an individual's health is complicated by the variety of potential infectious agents as well as the range of host responses. Unless disease symptoms are manifested, the interaction between microbial agent and host would pass unnoticed. Only by clinical observation can microbial infection be demonstrated, and then only if the correct analyses are being done. These qualifications must be considered in evaluating existing literature on the association of wastewater aerosols and disease.

Previous efforts to link wastewater exposure with human health effects have utilized a variety of observational approaches including retrospective and prospective studies at sewage treatment plants and wastewater irrigation sites. Using data collected between 1965 and 1971 as part of an intensive community health study, Fannin and associates (1980) evaluated the occurrence of acute gastrointestinal and respiratory diseases in families residing within 2400 m of an activated sludge treatment plant (1 MGD) in Tecumseh, Michigan. While persons living within 600 m of the plant had reported excess illnesses during summer months when compared to more distant households, the researchers concluded that this elevated illness rate was more likely to have been related to the high density of low socioeconomic families in that area rather than to the treatment plant.

Two prospective studies which utilized both clinical and environmental monitoring in areas around wastewater treatment plants have been reported. Johnson et al. (1980a) collected both baseline and operational year (9 months) data from families residing 350 m to 5 km from a new 30 MGD activated sludge treatment plant in Schaumburg, Illinois. Air sampling at the plant site showed that while indicator organisms were elevated, their numbers dropped to background levels at residential distances. Furthermore, enteric viruses were not detected in the air sampling. Self-reported illnesses as well as clinical microbial isolation and viral serology against 31 agents were used as tools to investigate the effect of wastewater aerosols on the study population. Although nearby residents reported a higher incidence of skin disease and gastrointestinal disorders during the operational year, virtually no serologic or clinical evidence was associated with proximity to the treatment plant. However, the pattern of echovirus 29 antibody response showed a slight association with aerosol exposure.

Working in a 1.6-km area surrounding an established sewage treatment plant (200 MGD), Carnow et al. (1979) conducted a similar study following a more intensive clinical sampling regime over an 8-month period. While aerosol sampling showed elevated fecal coliform counts within the plant,

downwind distances of 0.8 km and 1.6 km showed background levels of this indicator. No correlations were found between calculated exposure indices and the rate of self-reported illnesses or the microbial infection rates determined by agent isolation or antibody response.

Finally, an environmental monitoring program was coupled with an evaluation of retrospective school attendance records to investigate the potential health hazard posed by the operation of a new advanced wastewater treatment plant (approximately 10 MGD) located adjacent to an elementary school in Tigard, Oregon (Camann et al., 1980). The plant's aeration basin (approximately 400 m from classrooms and 250 m from the school playground) was noted as a source of indicator bacteria and coliphage, but no enteric viruses were detected. No overall effect of plant startup and operation was seen on school attendance relative to baseline school years and to five control schools. It was noted, however, that several periods of increased absenteeism occurred among the youngest students (first and second grade) after the treatment plant began operation.

Taken together, no definitive evidence can be found linking wastewater aerosol exposure to either illness or infection in the general population residing in areas around wastewater treatment plants in the United States. A similar conclusion was reached by Clark et al. (1981) after observing a population with high occupational exposure, namely sewer and sewage treatment workers and their families. In a 3-year prospective seroepidemiologic study involving workers in three metropolitan areas (Cincinnati, Chicago and Memphis), there was no consistent evidence for increased parasitic, bacterial or viral infections based either on agent cultivation or on antibody surveys. In a few instances, level of antibody to certain viruses in wastewater workers appeared to be related to level of exposure to wastewater aerosols. An increased level of minor gastrointestinal illness was noted during the spring season among inexperienced, sewage-exposed workers.

A study by Linnemann and coworkers (1984) of Muskegon County, Michigan, workers exposed to wastewater spray irrigation failed to show any differences in illness or viral isolation rates between the workers and a control group. Although antibody titers to coxsackievirus B5 were significantly higher in spray irrigation nozzle cleaners, seroconversions were not documented.

Aerosol exposure as a result of irrigation with wastewater provides yet another setting in which health effects on the surrounding community can be evaluated. An initial retrospective study in Israel implicated wastewater use in kibbutzim with an increased incidence of illness (Katzenelson et al., 1976). However, a more complete retrospective study of the incidence of enteric disease associated with wastewater utilization by kibbutzim in Israel (Shuval et al., 1983) raised serious questions about the results of the original study of Katzenelson et al. (1976). An excess risk of enteric disease was not associated with wastewater irrigation except in the 0-4 age group of kibbutzim of a ''switch'' category during periods of wastewater irrigation compared to periods during which wastewater was not used. This excess risk of total enteric disease ranged from 32 to 112% in this single group, a finding far different from the two- to fourfold increase of cases of salmonellosis, shigellosis, typhoid fever, and hepatitis reported from the kibbutzim practicing wastewater irrigation in the first study. Subsequently, Fattal et al. (1984) reported a prospective epidemiological study in 30 kibbutzim having varying degrees of wastewater utilization for irrigation. Paired sera were drawn approximately 1 year apart (1980-81) and tested for antibody to eight enteroviruses and varicella-zoster virus (as a negative epidemiologic control). Emphasis was placed on obtaining samples from young children (6 months to 5 years old) who would be more susceptible to viral infection. Serological results indicated that antibody to echovirus 4 was statistically more prevalent in kibbutzim practicing spray irrigation of wastewater within 600 m of the residential area (Category A) when compared to similar settlements in which wastewater irrigation was at a distance of ≥1000 m (Category B) or in which noneffluent water was used for irrigation (Category C). Notably, this increased antibody prevalence was observed in those Category A kibbutzim using wastewater from neighboring communities (as opposed to wastewater generated within the kibbutz itself).

Jakubowski (1983) has critically reviewed and evaluated previous wastewater health effects studies and has noted that the preponderance of data was negative. However, he observes that interpretation of the significance of the data, whether negative or positive, of all the studies is limited by the low numbers of highly exposed persons and the inability to adequately and quantitatively determine that exposure. None of the previous studies has investigated the health effects on residential populations exposed to sprinkler systems that apply wastewater to land according to EPA design criteria. The LISS was designed for this purpose and to answer many of the criticisms of previous studies, such as the reliance on self-reported illness, long-recall surveys or retrospective analysis of health data.

The LISS involved a variety of health watch activities including serology for viruses present in the wastewater, routine fecal specimens for bacteriological and virological analyses, analyses of illness specimens, tuberculin skin testing, household self-reports of illness and activity diaries. The health watch activities were supplemented by environmental monitoring of aerosols, wastewater, and drinking water. This study differs from previous U.S. studies in that, while both illness and infection were monitored, primary emphasis was placed on intensive infection surveillance.

Placed alongside these studies which used various epidemiological approaches to evaluate the effects of wastewater exposure on human health, the LISS has several unique attributes. The spray irrigation system at the Hancock farm was new, thus allowing baseline monitoring of the surrounding population. Once irrigation commenced, temporal exposure and infection data were collected over the course of multiple exposure/irrigation events. Perhaps more importantly, considering the positive findings of the Israeli study reported in 1984 and the lack of an association in the treatment plant studies, the wastewater used for irrigation on the Hancock farm was imported from a large metropolitan area. Thus, the LISS population was exposed to microorganisms circulating within another community, thereby increasing the likelihood of detecting an episode of infection introduced by wastewater irrigation. Another similarity between the Israeli studies and the first year of irrigation on the Hancock farm was the relative microbial strength of the wastewater sprayed directly from the pipeline during 1982. Finally, unlike the other health effects studies completed to date in the United States, aerosol sampling at the Hancock farm repeatedly demonstrated the presence of human viruses downwind of the spray source. Thus, it would appear that of the studies completed to date, the LISS was most likely to demonstrate a health response to wastewater aerosol exposure.

B. SUMMARY OF LISS FINDINGS

Wastewater spray irrigation at the Hancock farm commenced on February 16, 1982. The LISS monitored infection events and acute illness in the study population from July 1980 through September 1983 for possible association with irrigation.

Findings from Wastewater and Aerosol Data

The LISS monitored four major periods of wastewater irrigation at the Hancock farm. These periods were termed spring 1982 (February 16-April 30, 1982), summer 1982 (July 21-September 17, 1982), spring 1983 (February 15-April 30, 1983), and summer 1983 (June 29-September 20, 1983). The quality of the wastewater used for irrigation varied substantially by irrigation period. All of the irrigation wastewater was obtained via pipeline directly from the Lubbock SeWRP in the spring 1982 irrigation period, since operation of the reservoirs had not been approved at that time. The quality of this pipeline effluent was similar to that of a low quality primary effluent, as determined by physical and chemical analyses (see summary Table 21 and source Table P-1 in Appendix P). Pipeline wastewater comprised 64%, 0% and 1%, respectively, of the total applied by spray irrigation in the three following irrigation periods. There was some improvement in pipeline wastewater quality during summer 1982 and spring 1983, but it did not reach the quality expected of secondary effluent until summer 1982. Reservoir wastewater was more consistently of secondary effluent quality in all three of these periods. This observation is important, since the majority of irrigation wastewater used during 1982 came via pipeline directly from the SeWRP, while essentially all the wastewater applied during 1983 was from the irrigation reservoirs.

The wastewater utilized at the Hancock farm contained a broad spectrum of enteric bacteria and viruses. Spray irrigation of wastewater received via pipeline directly from the Lubbock SeWRP was found to be a substantial aerosol source of each group of microorganisms monitored in the aerosol sampling (i.e., fecal coliforms, fecal streptococci, mycobacteria, *Clostridium perfringens*, coliphage, and enteroviruses). Microorganism levels in air downwind of spray rigs using pipeline wastewater were found to be significantly higher than upwind levels: fecal streptococci levels to at least 300 m downwind, and levels of fecal coliforms, mycobacteria and coliphage levels to at least 200 m downwind. The downwind levels were also significantly higher than the background levels in ambient air outside the homes of participants: fecal coliform levels to beyond 400 m downwind, mycobacteria and coliphage levels to at least 300 m downwind, and fecal streptotocci levels to at least 200 m downwind. Operation at night and at high wind speeds appeared to elevate microorganism levels to greater downwind distances.

Enteroviruses were recovered in the aerosol at 44 to 60 m downwind of irrigation with pipeline wastewater on each of four virus runs. The geometric mean enterovirus density in air was 0.05 pfu/m^3, although a much higher density (17 pfu/m^3) was sampled on one run in August 1982. Spray irrigation of reservoir wastewater was also found to be source of aerosolized fecal coliforms, fecal streptococci and coliphage, sometimes to downwind distances of at least 125 m.

Since microorganism densities were much higher in the wastewater from the pipeline than from the reservoirs, the exposure which most of the study population received to most microorganisms via the wastewater aerosol was greater in 1982 than in 1983. The irrigation period in which aerosol exposure at a given distance downwind was estimated to be highest was: summer 1982 for enteroviruses, summer 1982 for fecal coliforms, and spring 1982 for fecal streptococci (see Table 42, using estimates for 150 to 249 m downwind when available). For each of the microorganism groups with adequate aerosol and wastewater monitoring data, summer 1982 was the irrigation period when most of the more highly exposed study population received either their largest or their second largest cumulative dose from the wastewater aerosol.

Findings from Self-reported Illness Data

Disease surveillance did not disclose any obvious connection between illness and degree of wastewater exposure. The self-reported illness data varied in consistency, reliability and completeness over the July 1980-September 1983 period of surveillance, with the better quality data obtained during the years of wastewater irrigation. In addition, self-reports of illness are always subject to respondent bias.

Nevertheless, it is of interest and may be significant that the participants in the high exposure level (AEI>5) reported the highest rate of illness shortly after the onset of wastewater irrigation, both in spring 1982 and in summer 1982. The excess total acute illness among high exposure level participants over the spring 1982 irrigation period occurred primarily during February 14-27, 1982, in the initial 2 weeks of wastewater irrigation at the Hancock farm. The extent to which this reflects actual illness as opposed to reporting bias by high exposure participants has not been ascertained. The high exposure level participants also reported a significant excess of total acute illness in August 1982, primarily during August 15-28 (after more than 3 weeks of wastewater irrigation had elapsed). The high exposure level participants did not report a comparable excess of acute illnesses during either irrigation period in 1983. This pattern of excess illness during both irrigation periods in 1982 is consistent with the hypothesis of an association of illness with exposure to wastewater irrigation: the pattern appeared both upon initial wastewater exposure and in the summer 1982 irrigation period which produced highest exposure to microorganisms in the wastewater aerosol. However, the patterns did not persist throughout either irrigation period in 1982. In addition, the effects of known risk factors such as age and socioeconomic status have not been taken into account. For total acute illness, the crude incidence density ratios of the high exposure level to the intermediate ($1 \leq AEI \leq 5$) and low (AEI<1) exposure levels were less than 1.5, both for the entire spring 1982 and summer 1982 irrigation periods. Thus, if not a reporting artifact, a small excess rate of illnesses might have been associated with the initial and heaviest periods of microorganism emission from wastewater irrigation. Since the agents which the LISS monitored clinically and serologically show a very high proportion of asymptomatic infection, it is difficult to correlate the findings for self-reported illness with those for the clinically and serologically detected infections.

Findings from Nonepisode Occurrences of Infections

The LISS detected the occurrence of a variety of infections which could not be analyzed as infection episodes. Many of these infections were detected in a nonsystematic manner (e.g., from illness or requested specimens) which precluded a determination of incidence for the study population. Other infections occurred too infrequently to constitute an infection episode. The results obtained from such occurrences of infection are summarized in Table 134 by infectious agent.

The occurrence of enteric Gram-negative bacteria (EGNB) at moderate and heavy levels in the throats of both healthy and ill study participants was both frequent and widespread between July 19 and October 12, 1982. This phenomenon was first identified in an extended illness investigation of a household in Wilson. The illness investigation established that the household environment was strongly associated with the continuing EGNB throat infections and identified the evaporative cooler as a potential source of infections. Among illness throat swab donors during the July 19-October 12 time period, use of an evaporative cooler for home air conditioning was associated ($p=0.02$) with the EGNB throat infections. A throat swab survey of healthy donors in September 1982 established an EGNB throat infection prevalence of 26% in healthy adults and teenagers at that time. The prevalence of these inapparent EGNB throat infections was higher in donors who frequently ate food prepared at restaurant A, who had high wastewater aerosol exposure, and whose homes used evaporative coolers for air conditioning. However, none of these potential risk factors were significantly associated with the inapparent EGNB throat infections.

Most of the infection occurrences presented in Table 134 appear to have been unrelated to wastewater irrigation. The highest or only period of occurrence of some infections was in the LISS baseline before irrigation commenced. In this category were *Yersinia enterocolitica* infections, nontuberculosis mycobacteria infections, and hepatitis A infections. *Entamoeba histolytica* infections occurred too infrequently to identify a period of higher incidence. The highest period of occurrence of other infections was between irrigation periods. The infections to Group A streptococci, *Salmonella*, EGNB in illness stools, and virus-like particles detected by EM in illness stools belong in this category. Other infections occurred primarily during an irrigation period, but in donors with lower average wastewater aerosol exposure (i.e., mean AEI) than the noninfected donors. In this category were the throat infections to Group A streptococci and EGNB among ill donors in summer 1982.

TABLE 134. SUMMARY OF FINDINGS PERTAINING TO POSSIBLE ASSOCIATION WITH WASTEWATER IRRIGATION FOR OCCURRENCES OF INFECTIONS NOT CLASSIFIED AS INFECTION EPISODES

Agent	Methods of observation	Period of greatest occurrence (O), prevalence (P) or incidence (I): period (rate, %)	Apparent association with wastewater aerosol exposure? (p-value)	Alternative explanation(s) (p-value)	References
BACTERIA					
Group A streptococci	Illness TS	O: Apr-Jun 1983 (31%)	No-between irrig. periods		Table 59
		O: Jul-Sep 1982 (24%)	No-lower mean AEI		
Salmonella	Illness investigation	O: Jun-Jul 1982	No (extremely unlikely)	Contam. drinking water (?), food (?)	Section 5.F
	RF	P: Jun 1982 (1%)	(see illness investigation)		Table 70
Other major enteric bacterial pathogens	RF	Y. entertocolitica, O: Jun-Jul 1982 (4%)	No-baseline		Tables 70 and 71
	RF	C. jejuni and Shigella not found	(No)		Table 70
	Illness fecal	None found	(No)		Table 64
Enteric Gram-negative bacteria (EGNB) (M or H level)	Illness investigation	O: Jul-Sep 1982	No (unlikely)	Evaporative cooler, Public swimming pool (?)	Table 68 Section 5.F
	Illness TS	O: Jul 19-Oct 12, 1982 (24%)	No-lower mean AEI	Evaporative coolers (0.02)	Tables 59 and 61
	Healthy donor TS survey	P: Sep 19-22, 1982 (26%)	Unlikely (0.18)	Eating at rest. A? (0.14) Evaporative coolers? (0.22)	Tables 62 and 63
	Illness fecal	O: May-Jun 1983 (36%)	No-between irrig. periods		Table 64
Non-tuberculosis mycobacteria (NTM)	Tuberculin skin tests	I: Jun 1980-Jun 1981 (2%)	No-baseline		Table 90
VIRUSES					
Viruses-isolates	Illness fecal	O: Jul-Sep 1982 (67%)	Unknown-insufficient data		Tables 64 and 66
Viruses-EM detections	Illness fecal	(None)	No-between irrig. periods		Tables 64 and 66
Hepatitis A	Serosurvey	I: Jun-Dec 1980 (0.3%)	No-baseline		Section 5.I
Coronavirus-like particles (CVLP)	EM of RF	O: Jul-Sep 1982 (18%)	Unlikely (0.12)-onset unknown		Tables 93 and 96
OTHERS					
Parasites	Serosurvey	E. histolytica (I): (<1%)	No		Section 5.I
	O&P survey-RF	G. lamblia (P): Jun-Aug 1983 (2%)	Unlikely, despite (0.03)	Household cluster Contam. drinking water	Tables 91 and 92
Repiratory illness following aerosol exposure	Illness investigation and RF	Aug 6-17, 1982	Possible (evidence consistent with aerosol hypothesis)	Person-person spread (?) Contaminated drinking water (?)	Section 5.F

TS - throat swab
RF - routine fecal specimens
EM - electron microscopy

There are insufficient data to determine whether other infection occurrences presented in Table 134 were associated with wastewater irrigation. Insufficient illness fecal specimens were obtained during the summer 1982 irrigation to determine if the mean AEI of donors with viruses recovered was higher than for the virus-negative donors.

Although not significantly associated (p=0.18), the inapparent EGNB throat infections detected in the September 1982 survey of healthy donors might be related to aerosol exposure. However, since the concurrent EGNB throat infections in ill donors were associated with evaporative cooler use at home, the evaporative cooler hypothesis may also be a more likely explanation for the inapparent infections (despite the lack of significant association with evaporative coolers: p=0.22).

The occurrence of coronavirus-like particles (CVLP) in routine fecal specimens in summer 1982 is unlikely to have been related to wastewater irrigation. The CVLP-infected donors had a higher average aerosol exposure than the EM-negative donors, but the difference was not significant (p=0.12). In addition, since many CVLP-infected donors were persistently positive, the onset of these CVLP infections may have preceded the summer irrigation.

The prevalence of Giardia lamblia in routine fecal specimens in summer 1982 is also unlikely to have been related to wastewater irrigation. All three of the five Giardia-positive donors who had high aerosol exposure were members of the same household. Since their Giardia infections cannot be considered independent, the apparently significant association (p=0.03) with wastewater exposure is invalid. The household's contaminated drinking water well or hand-to-mouth transfer of cysts are considered more probable routes of exposure.

The investigation of respiratory illnesses in children following aerosol exposure (see Section 5F) suggests a more likely association with wastewater irrigation than any of the other infection occurrences summarized in Table 134. Respiratory illnesses attributable via clinical isolates to coxsackievirus B4 and Achromobacter xylosoxidans were documented. Both of these agents were presumably present in the wastewater to which the ill children appear to have been exposed by spray irrigation of pipeline wastewater. The evidence of this illness incidence is consistent with the hypothesis that wastewater microorganisms transmitted by wastewater aerosol from spray irrigation infected and produced respiratory illness in the subject children. However, since plausible alternative modes of transmission such as person-to-person spread and contaminated drinking water were not investigated, the evidence for the aerosol exposure hypothesis is inconclusive.

Findings from Seroconversion Incidence Densities

An overview of the association of serologically detected infections with exposure to wastewater aerosols was obtained by comparison of the seroconversion incidence densities for serum donors in the three levels (or two groups) of aerosol exposure, for both the entire baseline (June 1980-January 1982) and the entire irrigation (January 1982-October 1983) periods of observations. The high exposure level participants had a higher incidence density of coxsackievirus B4 infections versus intermediate level participants during the entire irrigation period (see Table 84). In contrast, the high exposure level had no elevated infection incidence density to specific agents in the baseline period. Based on test-based 95% confidence intervals for the crude incidence density ratios, the high exposure group ($AEI \geq 3$) had a significantly greater incidence of infections to coxsackievirus B2 and echovirus 11 over the irrigation period, but a significantly greater infection incidence only to one agent, echovirus 9, during the baseline period (see Table 85). While extraneous variables were not investigated as alternative explanations, these results do appear to suggest an association between enterovirus infections and wastewater irrigation exposure.

A more sensitive analysis can be performed on groups of agents, provided the agent-person-time observations are independent. In the baseline period, the high exposure level had the lowest infection incidence densities of the three exposure levels to all of the adenoviruses tested, to all coxsackie B viruses tested, and to all echoviruses tested. In the irrigation period, the high exposure level had the highest incidence densities of infection by all coxsackie B viruses tested and by all echoviruses tested. Moreover, in the irrigation period the high exposure level also had the highest incidence density of infections to all of the tested viruses which had been recovered from the irrigation wastewater; the incidence density ratio of the high to the intermediate exposure level was significantly greater than 1.0 (see Table 86). Again, extraneous variables are not taken into account in this simplistic analysis. Nevertheless, these crude incidence densities suggest a probable association between seroconversions (especially to viruses recovered from the wastewater) and wastewater aerosol exposure. The crude incidence density ratios of the high exposure level to the intermediate and low exposure levels during the irrigation period were 1.8 and 1.5, respectively, for the viruses recovered from the wastewater, indicating some excess risk of viral infection from wastewater aerosol exposure.

Findings from Risk Ratio Scoring of Infection Episodes

A risk ratio score was assigned to each infection episode based on the infection incidence rates in the exposure levels and in the exposure groups (see Tables 100 and 101). The risk ratio score was symmetric with respect to the high and low exposure categories, with a positive score assigned if a pattern of excess infections occurred in the high exposure subjects and a negative score assigned if the same pattern of excess infections occurred in the low exposure subjects. Frequency distributions of risk ratio scores were formed for six jointly independent and mutually exclusive groups of infection episodes (see Table 102). For single and sporadic agents, the risk ratio scores of the control episodes (Group C) were symmetric about 0, as expected. However, there was a highly significant ($p=0.002$) excess of positive scores among exposure episodes whose duration spanned single irrigation periods (Group A) and a borderline significant ($p=0.09$) excess of positive scores among exposure episodes of 1-year duration (Group B). These results suggest that an excess risk of infection was associated with wastewater aerosol exposure. The seasonal distribution of positive scores in Group A was correlated with seasonal microorganism dose via aerosol exposure. The results from the risk ratio score distributions for grouped agents were similar. The risk ratio score approach provided evidence of a stable and dose-related association between infection events and wastewater aerosol exposure in the infection episodes observed by the LISS.

Findings from Confirmatory Statistical Analysis of Infection Episodes

The preliminary analysis found that the high (AEI≥3) and low (AEI<3) exposure groups were generally well balanced with regard to infection risk factors, including age, gender and previous titer. The high exposure group of serum donors had a significantly higher rate of polio immunizations during spring 1982. The high exposure group of fecal donors did contain significantly more farmers in the summer irrigation seasons. The high exposure fecal donors also ate food prepared at restaurant A very significantly more often in all four irrigation seasons. The exposure groups were stratified on polio infection status in comparing poliovirus seroconversion rates. No other stratification was done, because the number of observations was too small. After looking at the distribution of infected donors within households to investigate within household transmission, it was decided that the distribution in any single episode was not inconsistent with the hypothesis that the infections occurred independently in that episode.

A one-sided Fisher's exact test was employed in the confirmatory analysis (CA) to determine if the high exposure population had a larger infection incidence rate than the low exposure population. The test was applied to each agent in all exposure seasons for every agent which produced an infection episode in any of the seasons. The tests for association of infection incidence and wastewater exposure were significant at the α=0.05 level for seven infection episodes:

- CKLB4X--*Klebsiella* in summer 1983 (p=0.03)
- CWWI4X--clinical isolates of wastewater agents in summer 1983 (p=0.02)
- SCB25--coxsackievirus B2 in 1982 (p=0.05)
- SE090--echovirus 9 in baseline (p=0.02)
- SE246--echovirus 24 in 1983 (p=0.03)
- SPL11--poliovirus 1 in spring 1982 (p=0.02)
- SWWV5--seroconversions to wastewater isolates in 1982 (p=0.02)

The actual rate of positive associations in the exposure episodes appears to have been at least twice as large as the false positive rate. Among infection episodes involving single and sporadic agents, the positive rates were 4% in 27 independent control episodes (Group C), 6% in 31 independent single season exposure episodes (Group A), and 11% in 19 independent year-duration exposure episodes (Group B). For infection episodes involving grouped agents, the positive rates were 0/1 for the control episode, 1/8=13% for independent single season exposure episodes (Group D) and 1/2=50% for independent year-long exposure episodes. The actual rate of positive associations in control episodes was approximately equal to the expected false positive rate. In contrast, the actual rate of significant associations exceeded the false positive rate in each of the four independent groups of exposure episodes.

In conclusion, an excess of statistically significant associations of the presence of infection with wastewater aerosol exposure was found in the confirmatory analysis. The interpretation of the epidemiological importance of these significant associations must be moderated by recognition of the possibility that some of the tests may be significant only by chance and that some imbalances in the two poulations may provide alternate explanations for the observed differences. On the other hand, the number of detected increases in incidence rates associated with the wastewater irrigation may be underestimated, considering the relatively modest power of the tests to detect small differences. The certainty of the results is also lessened when the observational nature of the study and the difficulty inherent in determining appropriate assignment of individuals to the exposure groups are considered.

Findings from Exploratory Statistical Analysis of Infection Episodes

The exploratory logistic regression (ELR) analysis was conducted to investigate the association, if any, between presence of infection and degree of aerosol exposure (i.e., AEI), while controlling for the effects of other variables. Significant associations with AEI at a final step p-value below 0.05 were identified in four infection episodes:

- SE090--echovirus 9 in baseline (p=0.01)
- SPL11--poliovirus 1 in spring 1982 (p=0.01)
- SWWV2--seroconversions to wastewater isolates in summer 1982 (p=0.02)
- SSNV2--all seroconversions to serum neutralization-tested viruses in summer 1982 (p=0.04)

The significant covariates are presented in Table 125. The goodness-of-fit of each of these models was excellent.

The ELR analysis investigated alternative explanations to AEI (including eating food prepared at the restaurants in Wilson) for the infection episodes showing good or marginal evidence of aerosol exposure association by the four inferential methods employed. The alternative explanations identified are summarized in Table 133. Investigation of the route of wastewater exposure in the infection episodes where AEI was a significant predictor variable provided some evidence supporting all three routes (i.e., wastewater aerosol, direct contact with wastewater, and spending time in the irrigation environment on the Hancock farm). However, the aerosol exposure route received the most supporting evidence.

Evidence of Association of Specific Infection Episodes with Wastewater Aerosol Exposure

Specific infection episodes which displayed good or marginal evidence of association with wastewater aerosol exposure were identified by comparison of results from four methods of investigation (i.e., confirmatory statistical analysis, exploratory logistic regression analysis, confidence intervals of incidence density ratios, and risk ratio scoring). Additional evidence was considred regarding recovery of the infectious agent from the irrigation wastewater, seasonal correspondence of the infection response to aerosol dose, association with contaminated drinking water, alternative risk factors identified by ELR, and within-household transmission of infections.

A summary of this evidence was presented in Table 133 for each of the eight infection episodes with good or marginal evidence of wastewater aerosol exposure association. Any episodes in which a more plausible alternative explanation was not identified are more likely to have been causally related to wastewater aerosol exposure.

The eight infection episodes were placed in three categories based on the likelihood of causal association of the infection events with wastewater aerosol exposure:

1) More plausible alternative explanation identified:

   - Episode CKLB4X (*Klebsiella* infections in summer 1983)
     --alternative: eating food prepared at local restaurant A
   - Spurious control episode SE090 (echovirus 9 seroconversions in the baseline period)
     --alternative: within-household spread

2) Both aerosol exposure and identified alternative explanation(s) are plausible risk factors (evidence inconclusive):

   - Episode CVIR2W (clinical viral isolates excluding adenoviruses and immunization-associated polioviruses in summer 1982)
     --alternative: eating food prepared at local restaurant A
   - Episode SE115 (echovirus 11 seroconversions in 1982)
     --alternatives:
       - contaminated drinking water
       - caucasian, large household
   - Episode SWWV2 (seroconversions to viruses isolated from wastewater in summer 1982)
     --alternatives:
       - contaminated drinking water
       - low income, caucasian
   - Episode SWWV5 (seroconversions to viruses isolated from wastewater in 1982)
     --alternative: farmer, history of pneumonia
   - Episode SSNV2 (seroconversions in summer 1982 to all serum neutralization-tested viruses)
     --alternative: contaminated drinking water

3) Strong evidence of aerosol exposure association and no alternative explanation identified:

   - Episode SPL11 (poliovirus 1 seroconversions in spring 1982)

It should be noted that all five of the infection episodes in Category 2 relate to echo or coxsackie B viral infections observed primarily in summer 1982 and primarily to agents recovered from the wastewater at that time. Hence, it is reasonable to consider these to be five manifestations of a single nonpolio enterovirus episode centered on the summer 1982 irrigation season. With the heavy rainfall, rural drinking water contamination and other unusual circumstances which occurred during this summer, it is not surprising that fragmentary evidence of various alternative explanations surfaced for this nonpolio enterovirus episode.

There is strong evidence that the poliovirus 1 seroconversions in spring 1982 were associated with wastewater aerosol exposure. Furthermore, SPL11 is the only infection episode in which all four inferential methods provided evidence of a significant association. The Cochran-Mantel-Haenszel confirmatory analysis showed a significant association (p=0.02) of polio 1 seroconversions between January and June 1982 with the high aerosol exposure group in the spring 1982 irrigation, when controlling for the effects of polio immunizations during this time period. The groups were balanced regarding previous polio 1 titers. ELR selected polio immunization in spring 1982, low prior antibody level, and a high degree of aerosol exposure as strong predictor variables for SPL11 seroconversions in a well-fitting logistic model. Each variable may be considered a distinct risk factor for polio 1 seroconversions since each made a strong contribution to the ELR model. No alternative explanations to high AEI were identified by ELR. Poliovirus 1 was recovered three times from the pipeline wastewater sprayed in spring 1982. Therefore, the poliovirus 1 seroconversions in spring 1982 provide substantial evidence of a causal association with wastewater aerosol exposure.

C. COMPARISON OF FINDINGS TO THE LITERATURE

Self-reported Illness

Due to the paucity of prior data linking wastewater exposure to either microbial disease or infection, there is virtually no basis for evaluating the findings of the LISS relative to those previously described. The finding of excess self-reported illnesses among high exposure LISS participants after irrigation commenced is similar to findings observed in other studies (see Discussion in Section 6A). Although this in itself raises the suspicion of association with wastewater irrigation, it was difficult to evaluate epidemiologically and was not thoroughly analyzed biometrically. One problem is that the definition of illness varies from person to person. Last (1983) stated that, ''The words disease, illness and sickness are loosely interchangeable--but not wholly synonymous.'' M. Susser (1973) suggested the following definitions:

> Disease is a physiological/psychological dysfunction.
>
> Illness is a subjective state of the person who feels aware of not being well.
>
> Sickness is a state of social dysfunction, i.e., a role that the individual assumes when ill.

Therefore the self-reported information collected could be biased by the participants' attitude towards the project and perception of odor, as well as by personal situations that arose over the study period.

The monthly incidence density of total acute illnesses reported by the LISS participants varied from 2 to 13 per 1000 person-days observed. The National Health Interview Survey (National Center for Health Statistics, 1984) which also collected information on self-reported acute conditions through household interviews obtained an annual density of 6.3 acute conditions per 1000 person-days of inquiry in 1980-81. The density varied inversely with age in a nearly linear manner, from 8.8 for persons under 17 years old to 3.3 for persons over 65 years of age. Besides age, these rates varied inversely with family income. An additional consistency found in the LISS self-reported illness data that has been found repeatedly in a number of surveys (Fox et al., 1972; Elveback, et al., 1966; Monto and Koopman, 1980; Northrop et al., 1980) was the higher incidence of reporting respiratory illness than gastrointestinal conditions. Thus, it appears that the incidence of self-reported illness obtained from this study population was generally consistent with epidemiologic expectations of acute (including infectious) disease occurrence.

Given the inherent weaknesses associated with the collection of such data and the uncertainty surrounding biased reporting, it is not possible to draw firm inferences about wastewater irrigation health effects from the LISS data on self-reported acute illness. The resolution of wastewater-related health effects must rely on independent objective infection responses as measured by either isolation of infectious agents or serologic response.

Bacterial Agent Episodes

It was assumed that apparent disease might constitute only a small part of the total number of infections that might occur during wastewater irrigation. Thus, methods were designed to rigorously search not only for overt enteric bacterial pathogens such as *Salmonella*, *Shigella*, *Yersinia enterocolitica*, and *Campylobacter jejuni*, but also for heavy colonization by important opportunistic pathogens and for unusual occurrences of organisms which were prominent in wastewater but rare in fecal specimens from initial baseline monitoring.

Two major points must be emphasized that concern the approach and results of the bacteriological monitoring of health watch participants in the LISS. Firstly, we did not equate the term ''infection'' with ''disease,'' the latter being indicated by detectable alterations in normal tissue functions (i.e., clinical manifestations of illness). Infection was used in the broader sense of the entrance and multiplication of a microbe in the body. Secondly, the *health significance* of the organisms sought covered a wide spectrum, ranging from highly significant to little or no health significance. Organisms of three categories were chosen in order to provide a more sensitive indicator of possible wastewater risk, rather than disease, resulting from wastewater exposure.

The organisms of our first category, overt enteric pathogens, are of major clinical significance because they often are associated with disease and even inapparent or subclinical infections may provide a source for infection and disease in others. In spite of a rigorous search for overt enteric bacterial pathogens, the number of isolations from the routine fecal specimens was small in baseline monitoring (three) and periods after commencing of irrigation (one).

Thus, given the constraints of the size of the fecal donor population at risk, the results of this study do not appear to support an increased risk of acquisition of overt enteric bacterial pathogens associated with wastewater exposure. Relevant to this conclusion was the fact that overt pathogens often were detected in the wastewater sampling, with the exception of *Shigella*, which may have been below the level of detection by the direct plating and enrichment procedures used. Lack of infection by these organisms may have been due to failure to achieve an infectious dose through aerosol or direct contact. The size of inoculum required to produce disease in humans varies widely for enteric pathogens (Gangarosa, 1978), ranging, for example, from as few as 10 organisms for *Shigella* to 10^8 for most serotypes of *Salmonella*.

The clinical significance of fecal isolates of the organisms at levels defining the other two categories is questionable. However, opportunistic pathogens were infrequently isolated at levels defining Category 2 during baseline fecal sampling and only 0.3% of the baseline samples yielded isolates meeting the definition of Category 3. These observations coupled with the prominence of some of the organisms (particularly *Aeromonas hydrophila*, the fluorescent *Pseudomonas* group, and *Klebsiella pneumoniae*) in wastewater led us to believe that the two categories might provide a sensitive indicator of a possible health risk associated with exposure to wastewater. In addition, the organisms may be associated with enteric disease if isolated in large numbers from stools. For example, enterotoxin-producing *Klebsiella*, *Enterobacter*, *Proteus*, *Citrobacter*, *Serratia*, and *Aeromonas* have been isolated from the stools of children and infants with acute gastrointestinal symptoms (Wadstrom et al., 1976). Some *K*. *pneumoniae* and *Enterobacter cloacae* produce heat stable (ST) and heat labile (LT) enterotoxins, the latter of both organisms being immunologically related to cholera toxin and *Escherichia coli* LT (Klipstein and Engert, 1977). *K*. *pneumoniae* ST recently has been purified to homogeneity and found to have the same potency as *E*. *coli* ST in the suckling mouse assay and immunological cross-reactivity with the *E*. *coli* toxin (Klipstein et al., 1983). Likewise, *A*. *hydrophila* produces an enterotoxin, and the organism has been associated with diarrhea in American travelers, but not in Thais (Pitarangsi et al., 1982). A large percentage (41%) of *A*. *hydrophila* isolates from diarrheal stools were negative for enterotoxin in a recent study (Turnbull et al., 1984) and enterotoxicity was approximately equally divided (i.e., 58% and 53%) among fecal and environmental isolates. An interesting observation in the present study was that heavy levels of *Klebsiella* in feces and moderate or heavy levels of the prominent bacteria in wasteawater (primarily fluorescent *Pseudomonas* species) appeared to be associated with increased incidence and period prevalence of self-reported GI illness. Heavy levels of other opportunistic bacteria were not. It is apparent, however, that the quantitative importance of the organisms of Categories 2 and 3 in enteric disease is probably small and the etiological role of many of the organisms as enteric pathogens is not well established. Many of the organisms of Categories 2 and 3 do have unquestioned roles as major nosocomial pathogens (Guentzel, 1982).

A number of observations relating to nosocomial infections (NIs) by organisms of Categories 2 and 3 are perhaps relevant to the present study. The association of _Klebsiella_ infections with elderly males, albeit borderline significant, and the significant association of prominent wastewater bacterial infections with the elderly (see Table 72) may be related to the observation that the elderly are at increased risk for acquiring NIs. Gross et al. (1983) noted that of all NIs, 64% occurred after 60 years of age even though the elderly group represented only 23% of hospitalized patients. Increased prevalence and levels of intestinal colonization by organisms such as _Klebsiella_ in a hospital environment have been associated with severity of illness, duration of hospitalization, and use of antibiotics (Haverkorn and Michel, 1979; Goldmann et al., 1978; Selden et al., 1971).

At least six possible causes of the elevated levels of _Klebsiella_ and other opportunistic pathogens and the unusual isolations of organisms in Category 3 are suggested by the observations from NIs, other reports, and the present study. These include:

1) antibiotic selection of resistant organisms or promotion of growth as a result of reduction of competing flora by prior use of antibiotics,
2) ingestion of organisms on garden vegetables,
3) exposure to Gram-negative bacteria associated with heavily contaminated cotton,
4) fecal contamination of drinking water or food,
5) aerosols created by contaminated evaporative coolers,
6) wastewater irrigation operations.

Antibiotic selection or promotion of growth is an unlikely cause of the isolations in Categories 2 and 3 since the isolations were observed with routine rather than illness specimens. Ingestion of contaminated garden vegetables also is an unlikely cause, even though Wright et al. (1976) reported that salads may be heavily contaminated with Gram-negative bacilli. Wright et al. (1976) studied the flora of foods served to patients in a hospital and recovered enteric bacteria and _Pseudomonas aeruginosa_ from vegetable salads. The organism most frequently isolated was _Enterobacter agglomerans_ (85% of samples, 10^2-10^6 CFU/g). Other organisms isolated frequently and mostly at high counts were _E. cloacae_ (48%) and _Klebsiella_ (46%). The studies of Casewell and Phillips (1978) and Cooke et al. (1980) challenge some of the interpretations of Wright et al. (1976). Casewell and Phillips (1978) observed that food prepared for intensive care patients was frequently contaminated with _Klebsiella_ but noted that the hospital was the main source of contamination. Likewise, Cooke et al. (1980) examined hospital food for the presence of _Klebsiella_. Salads and cold meat were the most frequently contaminated foods. However, _Klebsiella_ also was widely distributed in the hospital kitchen environment which was considered, at least in part, to be the source of the organisms found in the food. It should be noted that *E. agglomerans*, the most frequent isolate from salads in the study of Wright et al. (1976), was isolated at any level of growth from less than 1% of the fecal specimens of LISS participants.

Exposure to Gram-negative bacteria associated with heavily contaminated cotton also is an unlikely cause of the unusual isolations. Morey et al. (1983) recently reported that seed cotton and cotton plants collected from Lubbock, Texas, were heavily contaminated with Gram-negative bacteria. The organisms were not identified; however, the investigators noted that *E. agglomerans* was the predominant species in other similar studies. *E. agglomerans* is a relatively recent designation for a group of organisms which include the former Herbicola-Lathyri bacteria which were included in the plant associated genus *Erwinia*. The nature of the flora of the contaminated cotton and the lack of relationship to the isolations from specimens of LISS participants make contaminated cotton an unlikely source. It should also be noted that while *K. pneumoniae* is widely distributed in the environment, strains isolated from humans and animals may be routinely different in properties. Bagley and Seidler (1977) noted that 85% (49/58) of *K. pneumoniae* of human and bovine origin were fecal coliform (FC) positive whereas 16% (19/120) of environmental strains were FC positive. Strains of *K. pneumoniae* that are FC positive have been shown to have other unique properties (Edmondson et al., 1980).

The fact that the unusual isolations of organisms of Categories 2 and 3 occurred over a defined period also tends to argue against possibilities 1 through 3, but not 4 through 6. Fecal contamination of drinking water as a consequence of contaminated individual wells and city of Wilson water is a possibility since most of the isolations occurred following a period of unusually heavy (>10 in.) rainfall in the study area in May and June 1982.

Klebsiella has been reported to be the most prevalent, potentially pathogenic Gram-negative bacterium in the air surrounding sewage treatment plants (Kenline and Scarpino, 1972; Randall and Ledbetter, 1966) and in air samples of wastewater used for spray irrigation (Linnemann et al., 1984). The organism also is found at very high levels in textile finishing plant effluents (Dufour and Cabelli, 1976) and in pulp and paper mill effluent discharge (Kanarek and Caplenas, 1981), and thus may be expected in the aerosols of those sources as well. Examinations of microorganism levels in air in the present study revealed unusually high levels of certain indicator organisms (fecal coliforms and fecal streptococci) that were carried long distances downwind from irrigation nozzle lines. These levels were greatest when irrigation was directly from the pipeline in the spring and summer of 1982. Presumably the aerosols also contained high levels of *Klebsiella*. However, *Klebsiella* infections were not associated with degree of aerosol exposure during this period of presumably greatest exposure in 1982.

Much of the interest in aerosols associated with sewage treatment and land application of wastewater has centered around small particle aerosols (i.e., 5 µm or less) which may be carried to deep areas of the lungs.

However, it has been proposed that most human bacterial pneumonia is due to microorganisms that have colonized the oropharynx, and that aspiration of such organisms may be the principal mechanism underlying nosocomial pneumonia (Sanford and Pierce, 1979). An interesting observation in experimental animals (mice and monkeys) was that K. pneumoniae administered by aerosol was significantly less virulent than when given by intranasal or intratracheal instillation (Berendt, 1978). These observations suggest that large particle aerosols containing the organism may lead to colonization of the nasopharynx associated with seeding of the gut by the organisms.

The use of evaporative coolers at home was identified as a potential source of infection by enteric Gram-negative bacilli (EGNB) at high levels in the throats of some health watch participants between July 19 and October 12, 1982. The authors are not familiar with studies describing transmission of EGNB, presumably via aerosolized particles, by this route. It is very unlikely that EGNB such as E. coli would be free living in the water or evaporative coolers. However, if fecal contamination of the well water used for this purpose had occurred, then this could be a potential source of infection. Given that the well water was contaminated, ingestion would remain quantitatively the most significant route of infection by enteric organisms.

An apparent association of Klebsiella infections with wastewater aerosol exposure occurred in summer 1983. However, frequently eating food prepared at restaurant A was more strongly associated with this infection episode and in the same individuals. The restaurant etiology may be more compelling for two reasons. Firstly, a part-time food handler at restaurant A was infected by Klebsiella during the same period, and secondly, the Klebsiella infections in summer 1982 were not associated with aerosol exposure, even though wastewater aerosol levels of Klebsiella were higher in summer 1982. However, the summer 1982 association could have been obscured by heavy rainfall-associated contamination of drinking water which occurred in that period.

In summary, the results of bacteriological analysis reported in this study dealing with the incidence of infection inferred by isolation of either overt or opportunistic pathogens from fecal specimens do not appear to suggest an increased risk associated with exposure to wastewater.

Viral Agent Episodes

Human viruses cannot replicate outside a susceptible host and hence their concentration in wastewater decreases due to dilution and eventually inactivation. However, the relative environmental stability of numerous enteric viruses shed into wastewater by infected individuals enhances their potential transmission to susceptible populations by wastewater aerosols. Dispersion modeling developed by Camann (1980) and based on limited data collected at a wastewater irrigation site in Pleasanton, California, predicted that median impact factors reflecting enhanced organism survival were approximately 20 times greater for viruses when compared to even the hardiest indicator bacteria (fecal streptococci). Indeed, aerosol monitoring during 1982 LISS irrigation periods repeatedly detected human enteroviruses in downwind air samples. Enterovirus survival in Hancock farm aerosols from pipeline wastewater irrigation was at least as great as that observed at Pleasanton.

In addition to their relative stability, the minimal infectious dose of various human enteric viruses is low when compared to most pathogenic bacteria found in treated wastewater (Akin, 1983). A comprehensive review by Ward and Akin (1984) evaluated numerous studies directed at determining the infectious doses of both respiratory and enteric viruses. The 50% human infectious dose (HID_{50}) for respiratory agents such as coxsackievirus A21 and adenovirus type 4 in aerosols was reported as 34 and 0.5 $TCID_{50}$, respectively. Notably, the dose of coxsackievirus A21 required to cause illness was apparently less when the infectious agent was delivered to the upper respiratory tract than when the virus was delivered to the lower portion of the gastrointestinal tract.

Infectious dose studies with enteric viruses known to replicate in human intestinal cells have been limited to polioviruses and echovirus 12. Without exception, poliovirus studies have measured infections in infants and young children, representing perhaps the most highly susceptible population. In one such study 2-month-old infants were fed doses of 7 to 280 $TCID_{50}$ of attenuated poliovirus 1 (Sabin) (Minor et al., 1981). Based on viral shedding the HID_{50} was determined to be 72 $TCID_{50}$. Earlier studies with polioviruses 1 and 3 which introduced the virus either directly into the stomach or employed gelatin capsules to transport viruses to the intestinal tract had demonstrated HID_{50} of less than 10 $TCID_{50}$ or pfu, respectively (Katz and Plotkin, 1967; Koprowski et al., 1956).

Healthy male subjects (18-45 years of age) initially lacking detectable antibody to echovirus 12 were challenged with various doses of this virus suspended in drinking water (Schiff et al., 1984). The HID_{50} of echovirus 12 was determined to be 919 pfu while the HID_{01} (dose required to infect 1% of the volunteers) was predicted as 17 pfu. In this study most viral shedding occurred during the first week after inoculation, regardless of the viral dose. The duration of viral shedding (up to 28 days) was also independent of dose. In a second experimental challenge in individuals seropositive for echovirus 12, 72% became reinfected (as determined by detection of virus in stool specimens) when 1500 pfu (HID_{60}) were ingested. Thus, the presence of serum antibody caused no significant change in the number of volunteers infected with echovirus 12.

Considered as a whole, response to infection by viral agents as measured either by fecal shedding or seroconversion probably provides the most sensitive measure of wastewater aerosol exposure currently available. Thus, the serological identification of discrete infection episodes occurring mostly in 1982 is feasible. Furthermore, these LISS findings are consistent with conclusions reached by Fattal and coworkers (1984) who suggested a wastewater exposure route for infection by echovirus 4.

However, further analyses of viral infection episodes as well as other infections possibly associated with wastewater exposure have identified alternative explanations in selected cases (see Tables 133 and 134) which should be weighed in the light of epidemiological consistency. Intrafamilial transmission of enteroviruses and adenoviruses has been well documented (Fox and Hall, 1980). In the New York virus watch program, the spread of coxsackieviruses to susceptible household members was high (76%) while echovirus transmission was somewhat lower (46%). Notably, while larger families of lower socioeconomic status yield enteroviruses more frequently, intrafamilial spread appears to be independent of family size. A more important correlation of infections among family members has been shown to be the duration of fecal shedding by infected individuals. Reinfection by both coxsackieviruses and echoviruses, even in the presence of specific antibody, also occurs (Fox and Hall, 1980; Schiff et al., 1984). A similar pattern of transmission between family members has been observed with adenoviruses in both the New York and Seattle virus watch programs (Fox and Hall, 1980). However, because of the relatively prolonged and intermittent excretion of adenoviruses, long continuing intrafamilial spread is not uncommon. For these reasons, the alternative explanation of within-family spread as applied to infection episode SE090 attributed to echovirus 9 (three of eight infections) should reasonably supercede the association of these events with wastewater exposure.

Ingestion of contaminated drinking water from private and public wells has been documented in several outbreaks of viral disease in the United States including hepatitis A, Norwalk virus and rotavirus (Bergeisen et al., 1985; Olivieri, 1984; Hopkins et al., 1984). Presumably, subclinical infections with other enteroviruses having similar environmental stability can occur, particularly if drinking water wells were contaminated with wastewater from septic tanks.

The involvement of selected enteric viruses in common-source foodborne disease outbreaks has been well documented. Cases of hepatitis A traced to the consumption of shellfish harvested from contaminated coastal waters is well known. Additionally, ingestion of uncooked or cold foods such as salads (Latham and Schable, 1982), meats and cheeses (Gustafson et al., 1983) have been linked to hepatitis A outbreaks. However, of the 1,097 confirmed foodborne outbreaks reported to CDC between 1972 and 1978, only 3% were attributed to viruses, while 66% were due to bacteria (Sours and Smith, 1980). Twenty-nine of these viral outbreaks accounting for 1,346 cases were attributed to hepatitis A, while a single outbreak caused by echovirus 4 involved 80 cases. Thus, while poor personal hygiene of a food handler can cause the viral infections of restaurant patrons, relatively few foodborne outbreaks of viral etiology have been documented.

The remaining alternative explanations identified in Tables 133 and 134 for viral infections were race (caucasians) and previous medical history (pneumonia). In studying the response within households to poliovirus infection, Fox and Hall (1980) noted that socioeconomic group showed a greater influence on the percentage of individuals with specific antibody than did race. Specifically fewer of the whites in an upper economic group had neutralizing antibody than blacks and whites in the lower economic group who developed parallel seroimmunity to poliovirus with increasing age. Previous disease occurrences, especially if tissue damage resulted, can predispose an individual to subsequent infection by viral agents.

D. SIGNIFICANCE OF FINDINGS

Assessment of the significance of the findings from the LISS requires that particular attention be given to the possible limitations of the study. The design employed, an epidemiologic analytic cohort study, was quite appropriate to measure the strength of association between exposure to the wastewater used for irrigation and the development of new infections. As a guide to the following discussion, the more frequent, important limitations that may occur with the prospective cohort study design are presented first, followed by the major advantages of this design. Then, the specific limitations of the LISS are presented and discussed.

A major limitation in interpreting the strength of association, i.e., relative risk, from this type of study design can arise from bias introduced by uncontrolled confounding factors. Another limitation may be imposed by instability of the association when the sample size is small. By using consenting study participants, the findings may be inferred to the study population only with caution, since volunteer populations are known to differ from nonparticipants in risk factors related to viral infections (Francis et al., 1955). Unless the study population were representative of the general situation involving exposure to wastewater for irrigation purposes, it would be unwise to generalize from the LISS findings. Finally, bias may be introduced during ascertainment of the study variables due to missing values or transcription errors or the methods employed for measuring may produce misclassifications.

If these limitations are either prevented or controlled, the prospective cohort design may have several important strengths in assessing causality of associations. Since this is a study of incidence, exposure is known to precede infection. The hypothesis of causal inference may be strengthened by: a strong association that is stable, the demonstration of a dose-effect, an association that is consistent at different times, an association in agreement with biologic and epidemiologic theory, and an association which is specific.

A major limitation in interpreting the significance of the findings from the LISS involves the selection of participants. Of necessity all participants were volunteers. The study sample was not representative of the study population. Further, we can only assume that self-reported illness was accurately reported during the study. The source of irrigation wastewater varied during the study, making interpretation of findings difficult, since the dose of exposure varied within the exposure levels by irrigation period. Because of these factors, the results cannot easily be generalized to other sites.

The preliminary analysis compared the low exposure group and the high exposure group with respect to several individual and household characteristics that could confound the interpretation of the significance of the findings. Of the six variables considered important enough epidemiologically to warrant stratification for an imbalance, only polio immunization and fecal donor head of household occupation met the criteria for stratification. For the serum donor sample, type of air conditioning and drinking water supply were found to be different. Preexisting antibody titers to only three agents (influenza A in June 1981, echo 3 in January 1982 and polio 3 in January 1982) were not balanced. The exposure groups were significantly imbalanced with respect to frequency of eating food prepared at restaurant A. In general, the two exposure groups were quite similar in risk factors that could confound interpretation of the relative risk. Poliovirus seroconversion rates were stratified on polio immunization status. Sample size was too small to permit stratification for air conditioning, drinking water supply and patronage of restaurant A. Therefore, each relative risk analysis with a value greater than 1 had to be reviewed with these characteristics as an alternative explanation. Exploratory analysis using a stepwise logistic regression model served this purpose (except for air conditioning system in 1982). The significance of the study findings have not been limited to a great extent by the major confounding factors.

The size of the study sample has limited the ability to interpret the stability of the strength of association in most instances. Therefore, it was necessary to rely more on the consistency of the findings. The three outcome variables selected for the study varied in sensitivity to detect infection, ranging from low sensitivity for clinical disease, intermediate sensitivity for infectious agent isolation, to high sensitivity for serologic determination of infection. The self-reported illness data of the disease surveillance varied in consistency, reliability and completeness, which makes interpretation difficult. High but unstable incidence density ratios of acute illness for the high exposure level followed wastewater irrigation in the spring and the summer of 1982. According to the aerosol results, microorganism dosage was greater in 1982 than 1983, with the summer of 1982 being greatest for enterovirus exposure. Disease surveillance did not disclose any obvious consistent association between acute illness reports and the degree of wastewater exposure.

The results from isolation and serologic determination are more reliable and accurate. During the baseline period the high exposure group had the lowest conversion rates to all the adenoviruses, coxsackie B viruses, and echoviruses tested; however, in the irrigation period the high exposure group had the highest seroconversion rates to all coxsackie B viruses tested, to all echoviruses tested, and to all the tested viruses recovered from irrigation wastewater. The risk ratios were greater than 1 but less than 2. When the risk ratio scores of each infection episode were displayed graphically, the baseline distribution was symmetrically centered about zero; however, an excess of positive scores occurred in the episodes whose duration spanned single irrigation periods.

Using the one-sided Fisher's exact test in the confirmatory statistical analysis revealed seven infection episodes with stable risk ratios. When the results were compared using the four statistical approaches, eight specific infection episodes were identified which displayed marginal to good evidence of association with wastewater aerosol exposure. The two episodes which had a more plausible alternative explanation occurred in the baseline period and during the summer of 1983. Of the remaining six episodes, all occurred during 1982 and one episode had strong evidence of aerosol exposure association.

Except for illness ascertainment, the results from isolation and serology appear to be adequate. There is no evidence that the results were biased by additional efforts in detection. The laboratory methods would underestimate infections in general, but not by exposure group. Classification of participants into exposure groups was done employing a reasonable model which estimated exposure level by distance from the irrigation sprayers, wind direction, and risk of direct contact. Review of participants revealed no significant classification error.

In summary, the results indicate that a general association between exposure to irrigation wastewater and new infections existed, especially for 1982. However, even during 1982, the strength of association remained weak and frequently was not stable. Wastewater, directly from the pipeline, comprised much of the irrigation water in 1982. The isolation of enteroviruses from pipeline wastewater was greater than that observed when the wastewater had been retained in reservoir. The methods employed resulted in the observation of a large number of infection episodes, none of which resulted in serious illness. The voluntary nature of participation and the unrepresentative circumstances of the study area make generalization of the results unwise. A larger sample size with greater comparability of the exposure groups on the basis of drinking water source and frequency of visiting the same eating establishments would have reduced their confounding effects.

From the public health standpoint, the lack of a strong, stable association of clinical illness episodes with the level of exposure to irrigation wastewater indicates that wastewater spray irrigation produced no obvious disease during the study period. However, when more sensitive indicators of infection were used, a general association was found to exist, especially for 1982. A particular concern is the evidence that the poliovirus 1 seroconversions were probably related to wastewater aerosol exposure during the spring of 1982, even when the effects of polio immunizations were controlled. Because of the low prevalence of poliovirus antibody observed during the baseline period, the study population had been immunized, and thus was probably better protected against polio than other rural populations. Very high concentrations of both bacteria and enteric viruses were observed in the 1982 wastewater applied as received via pipeline directly from the Lubbock sewage treatment plant. Much lower concentrations were observed in wastewater obtained from the reservoir. Although the LISS found no obvious evidence that disease was associated with using treated wastewater for irrigation during the study period, as a public health measure it would be prudent to allow the wastewater to settle in a reservoir before use if other conditions remain the same.

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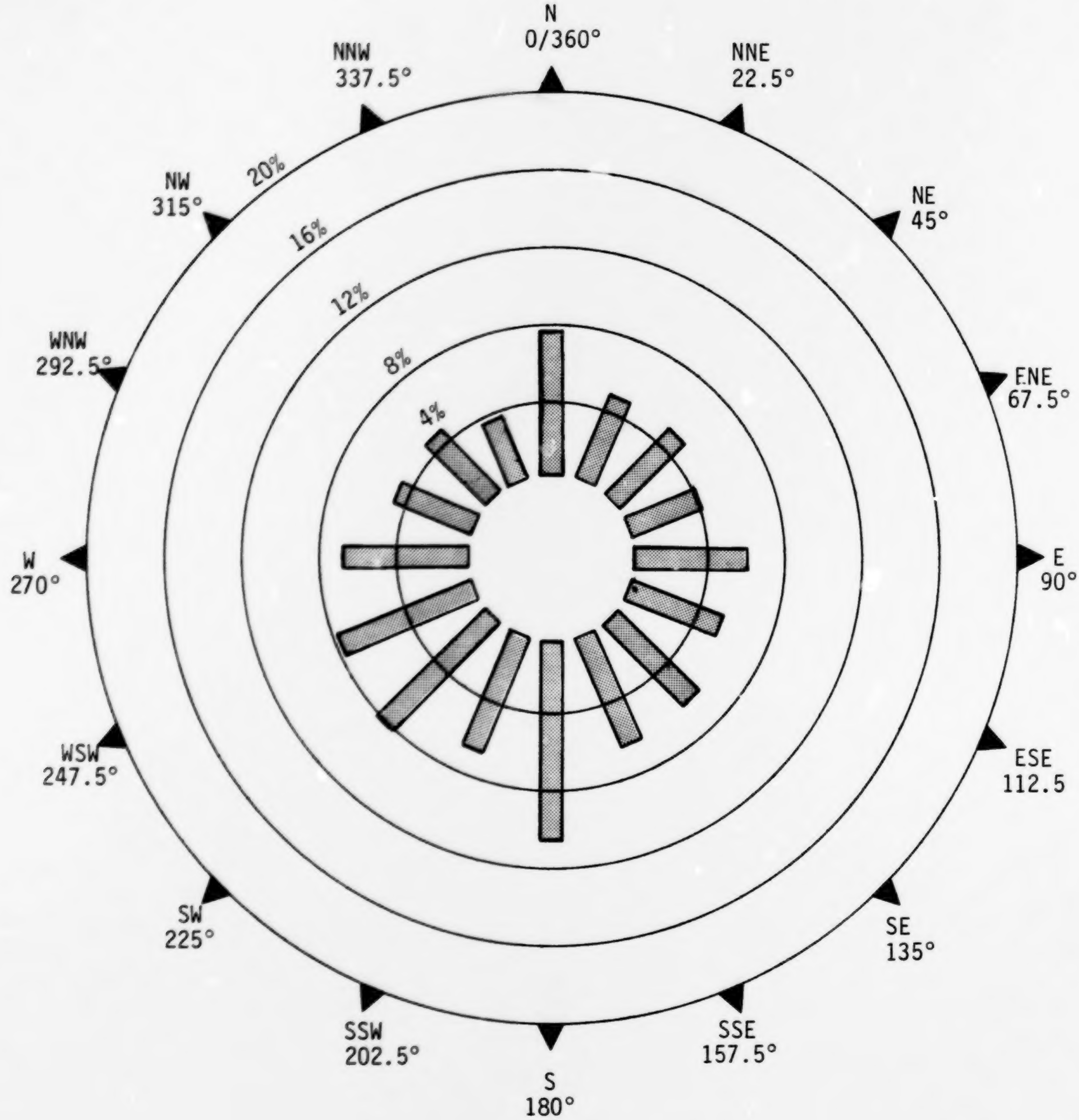
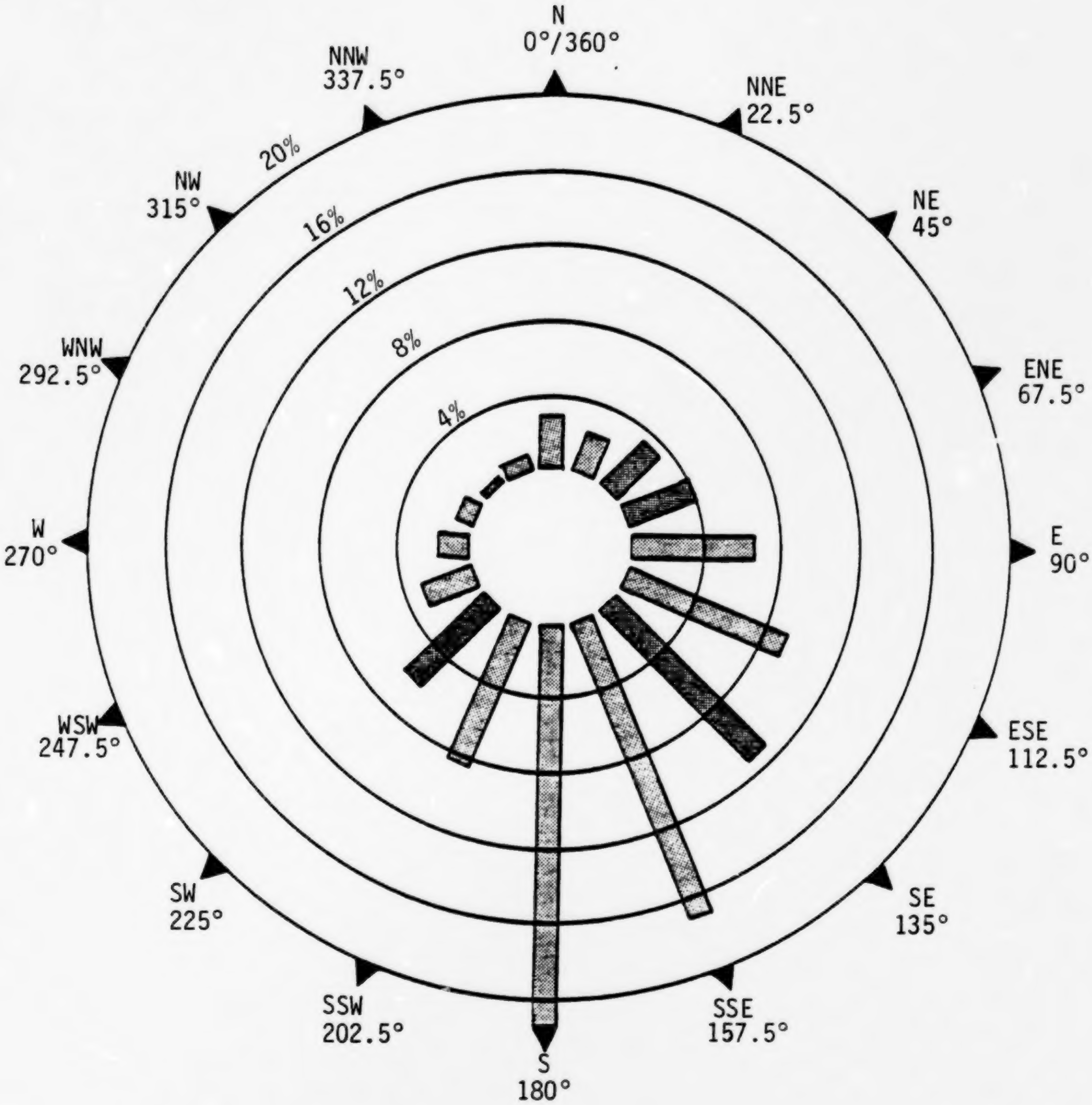
APPENDIX A

SUPPLEMENTAL FIGURES AND TABLES FOR SECTION 4
(METHODS AND MATERIALS)



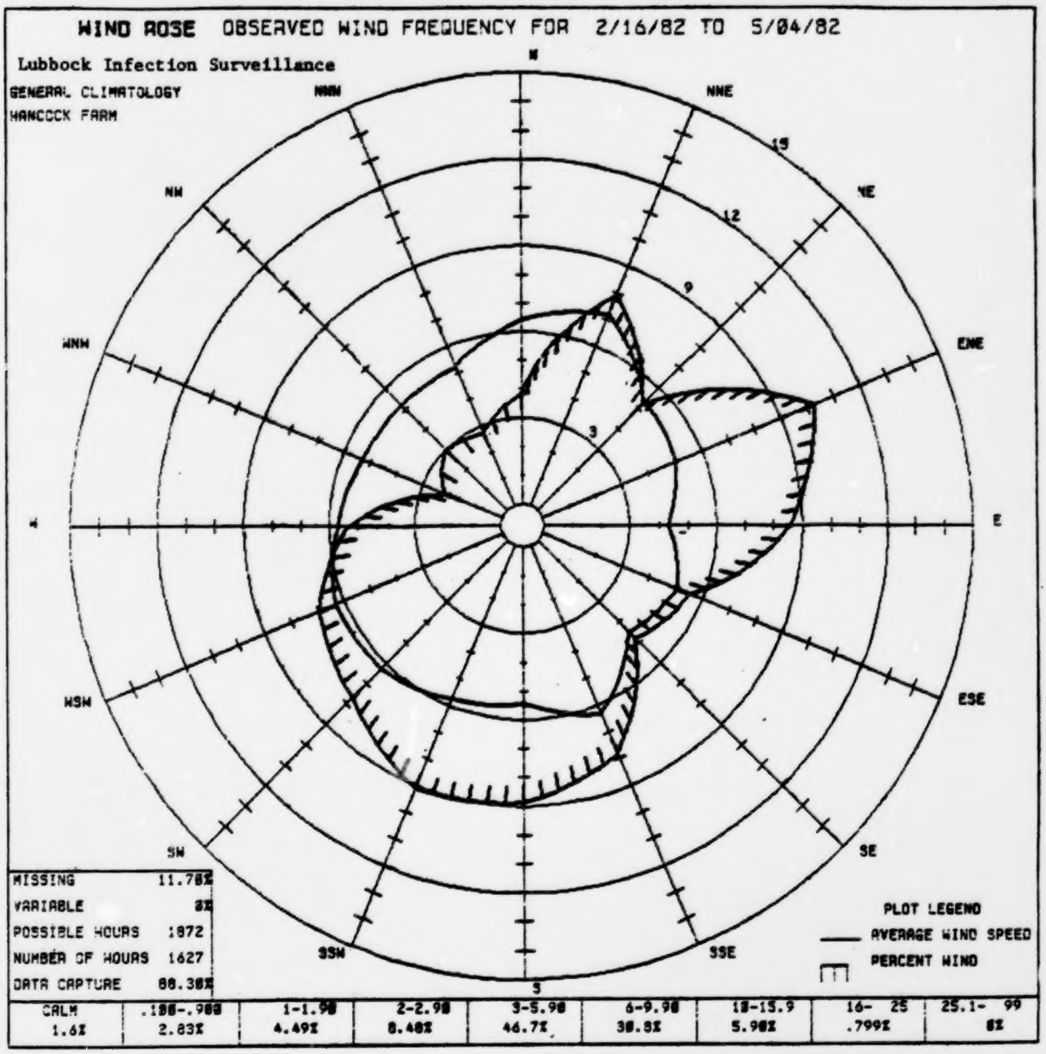
NOTE: Three-hour observations are from the 5-year period, 1969-1973. Radiating-bar lengths indicate the percent of the period that winds blow from the indicated directions.

Figure A-1. Wind frequencies for the 2-month period of March-April, Lubbock, Texas



NOTE: Three-hour observations are from the 5-year period, 1969-1973. Radiating-bar lengths indicate the percent of the period that winds blow from the indicated directions.

Figure A-2. Wind frequencies for the 2-month period of July-August, Lubbock, Texas



MISSING	11.70%
VARIABLE	0%
POSSIBLE HOURS	1872
NUMBER OF HOURS	1627
DATA CAPTURE	88.30%

CALM	.100-.900	1-1.90	2-2.90	3-5.90	6-9.90	10-15.9	16- 25	25.1- 99
1.6%	2.83%	4.49%	8.40%	46.7%	30.8%	5.90%	.799%	0%

Figure A-3. **Wind frequencies for the 1982 spring irrigation period: Hancock farm meteorological station**

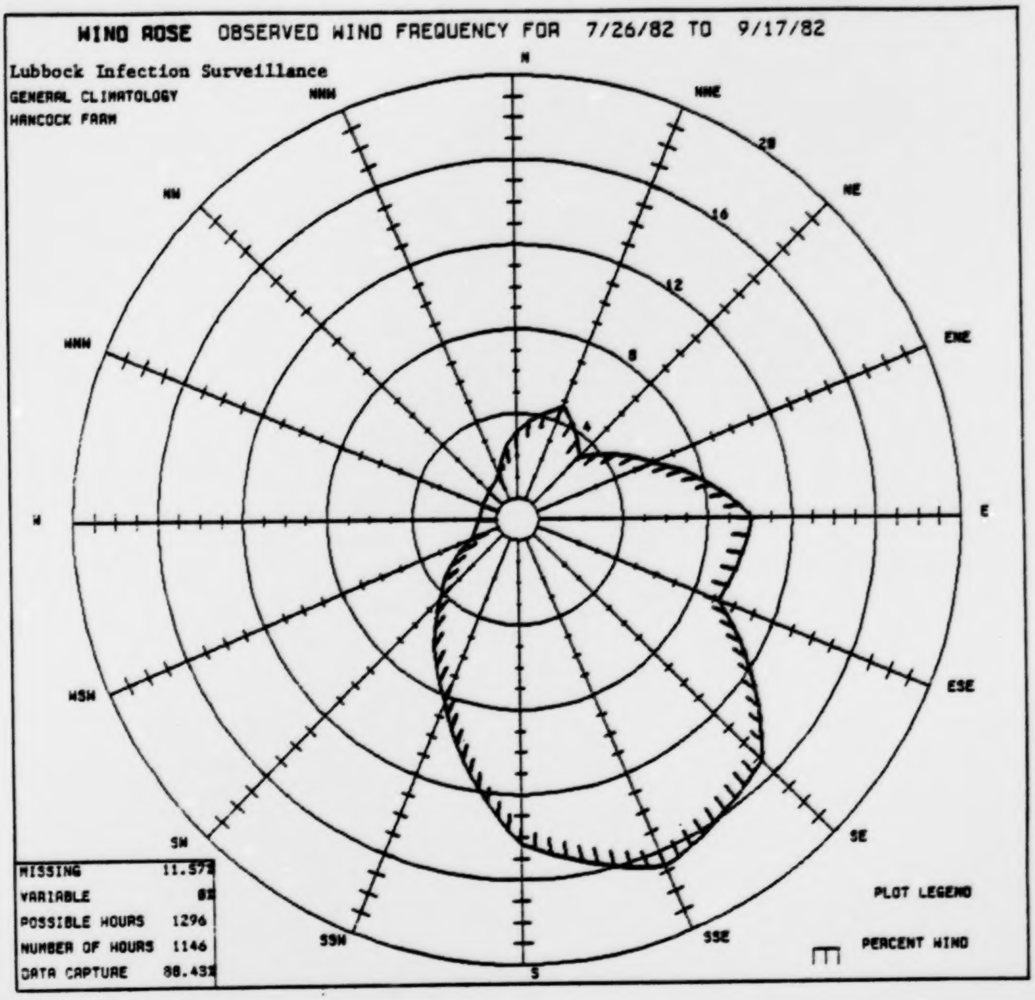
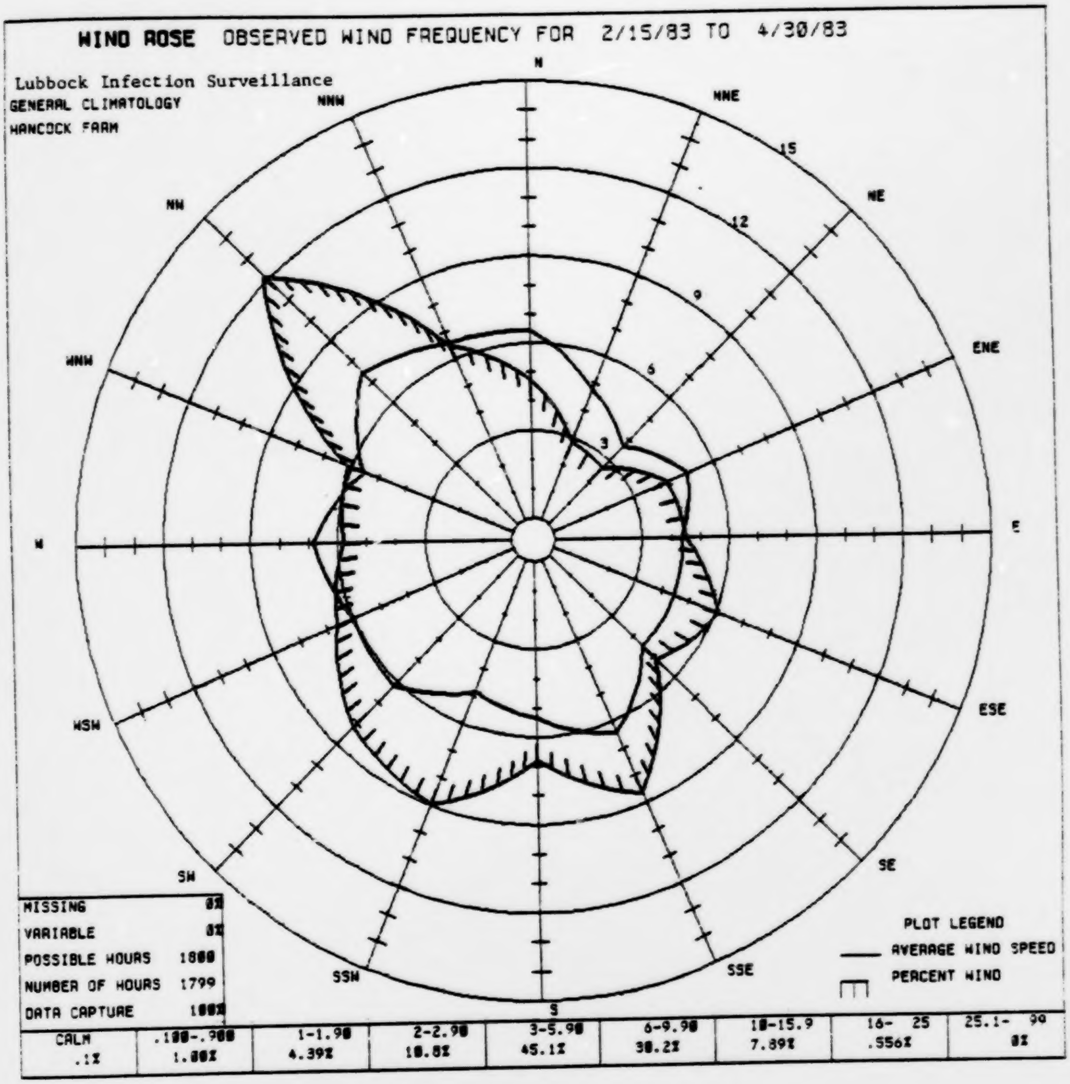


Figure A-4. **Wind frequencies for the 1982 summer irrigation period: Hancock farm meteorological station**


WIND ROSE OBSERVED WIND FREQUENCY FOR 2/15/83 TO 4/30/83

Lubbock Infection Surveillance
GENERAL CLIMATOLOGY
HANCOCK FARM

MISSING	0%
VARIABLE	0%
POSSIBLE HOURS	1800
NUMBER OF HOURS	1799
DATA CAPTURE	100%

CALM	.100-.900	1-1.90	2-2.90	3-5.90	6-9.90	10-15.9	16- 25	25.1- 90
.1%	1.00%	4.39%	10.8%	45.1%	30.2%	7.89%	.556%	0%

Figure A-5. **Wind frequencies for the 1983 spring irrigation period: Hancock farm meteorological station**

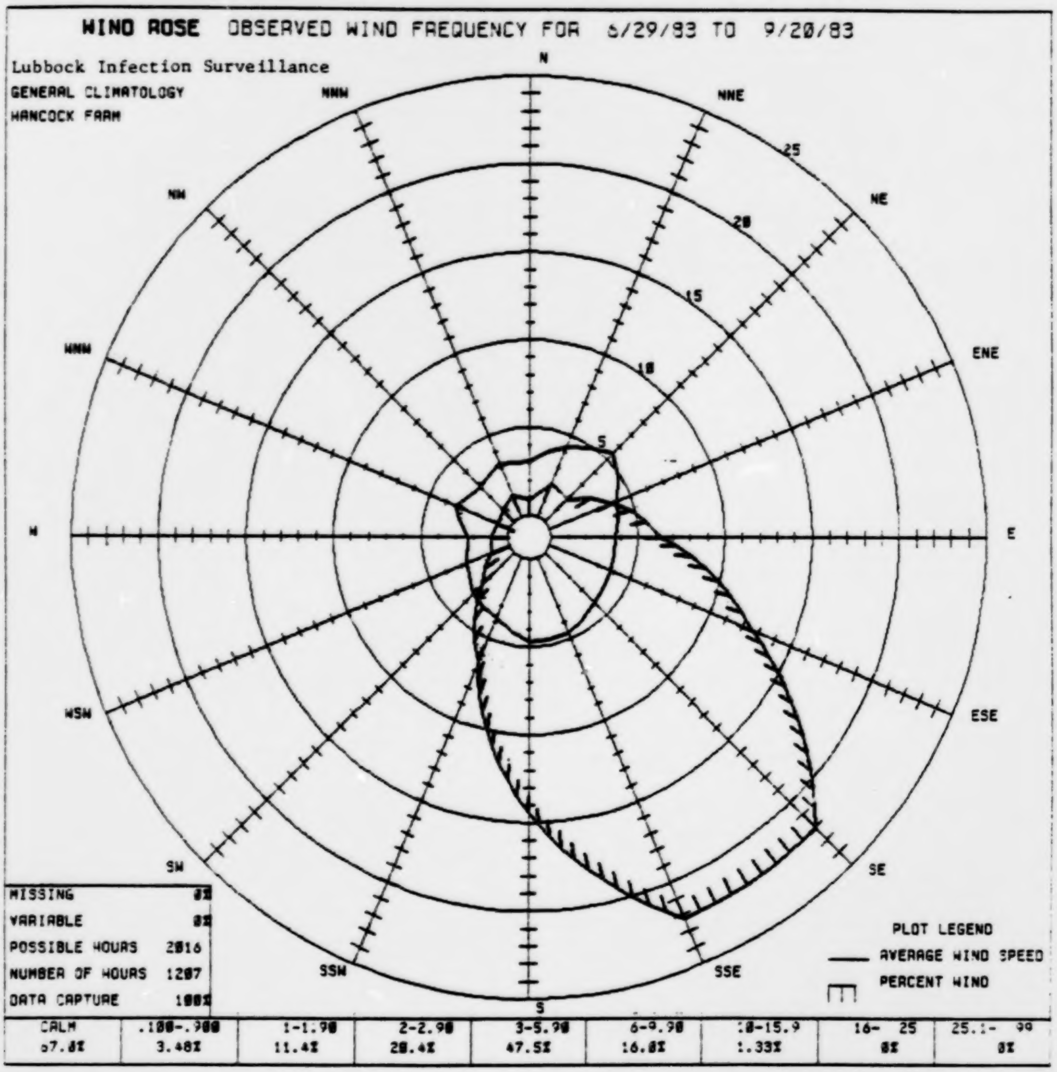


Figure A-6. **Wind frequencies for the 1983 summer irrigation period: Hancock farm meteorological station**

TABLE A-1. VALUES OF PREDICTED RELATIVE AEROSOL CONCENTRATION, P_d

Irrigation period and dates of irrigation	1 2-16/4-30-82	2 7-21/9-17-82	3 2-15/4-30-83	4 6-29/9-20-83
Activity diary map (1965-1974 wind data)	Feb-Apr	Jul-Aug	Feb-Apr	Jul-Aug
Range of household values, P_h[a]	0.00004-0.19	0.00003-0.30	0.00004-0.19	0.00004-0.30
Blue map area (Hancock farm)	$\bar{P}_1 = 0.1207$	$\bar{P}_1 = 0.1806$	$\bar{P}_1 = 0.1207$	$\bar{P}_1 = 0.1806$
Orange map area (surrounding Hancock farm)	$\bar{P}_2 = 0.0244$	$\bar{P}_2 = 0.0221$	$\bar{P}_2 = 0.0243$	$\bar{P}_2 = 0.0219$
White map area (remainder of study area)	$\bar{P}_3 = 0.0011$	$\bar{P}_3 = 0.0017$	$\bar{P}_3 = 0.0012$	$\bar{P}_3 = 0.0017$
Outside map area	$\bar{P}_4 = 0$	$\bar{P}_4 = 0$	$\bar{P}_4 = 0$	$\bar{P}_4 = 0$

NOTE: $\bar{P}_1$, $\bar{P}_2$ and $\bar{P}_3$ are geometric means of P_h values of all study participant households in the respective colored map areas.

[a] P_h = predicted rlative aerosol concentration at participant's home

TABLE A-2. WASTEWATER SAMPLING AND ASSAY SCHEDULE: 1980-81

	Lubbock trickling filter effluent			Wilson Imhoff tank effluent		
Sampling dates	Full microbiological screen	Limited bacterial screen	EV and FC	Full microbiological screen	Limited bacterial screen	EV and FC
1980						
6-3/6-4	x	x	x0	x		x0
7-28/7-29	x	x	x0	x		x0
11-3/11-4	x	x	x0			
1981						
1-19/1-20			x			x
2-16/2-17			x			x
3-9/3-10			x			x
3-23/3-24		x	x			x
4-20/4-21	x	x	x0			x
5-4/5-5		x	x			x
5-18/5-19						x
6-1/6-2						x
6-15/6-16		x	x0			x0
6-29/6-30		x	x			x
7-20/7-21	x	x	x0		x	x
8-17/8-18		x	x0		x	x0
9-14/9-15					x	x
11-17/11-18		x	x		x	x

x - performed on composite wastewater sample from designated source
O - viral identification performed on this sample
EV - enterovirus assay
FC - fecal coliform assay

TABLE A-3. WASTEWATER SAMPLING AND ASSAY SCHEDULE: 1982

	Pipeline effluent				Reservoir effluent					Wilson effluent	
Collection date	Full microbiological screen	Limited bacterial screen	Routine assay[a]	EV and FC	Sample type[b]	Full microbiological screen	Limited bacterial screen	Routine assay[a]	EV and FC	Limited bacterial screen	EV and FC
2-15/2-16	x^L	•	•	•						x	x
3-1/3-2		x	x	•							x
3-8/3-9		x	x	•O						x	xO
3-15/3-16			x	•							
3-22/3-23	x^L	•	•	•O						x	x
3-29/3-30			x	•							
4-5/4-6		x	x	•O						x	xO
4-19/4-20		x	x	•O							x
4-26/4-27			x	•							
5-2/5-3											x
5-17/5-18											x
6-14/6-15			x	•	G			x	•		x
6-29/6-30		x^L	x	•O	G		x^L	x	•	x	xO
7-19/7-20										x	x
7-26/7-27	x^L	•	•	•O	C	x^L	•	•	•		
8-9/8-10		x		x	C		x		x	x	xO
8-30/8-31[c]		x	x	•	C		x	x	•	x	x
9-13/9-14		x	x	•O	C		x	x	•	x	xO
9-27/9-28										x	x
10-11/10-12										x	x
11-1/11-2			x		G			x			x
12-13/12-14			x		G			x			x

x – wastewater sample collected for indicated assay
• – assay performed as subset of another assay
O – viral identification performed on this sample
x^L – Legionella assay performed in addition to regular assay

EV – enterovirus assay
FC – fecal coliform assay

a Same organisms monitored on aerosol runs (fecal coliform, fecal streptococci, coliphage, total enteroviruses, and C. perfringens/mycobacteria).
b C – composite sample; G – grab sample.
c Chlorination of pipeline effluent of Lubbock wastewater treatment plant.

TABLE A-4. WASTEWATER SAMPLING AND ASSAY SCHEDULE: 1983

Collection dates	Pipeline effluent			Reservoir effluent				Wilson Imhoff influent
	Routine assay[a]	Coliphage	Limited screen	Sample type[b]	Routine assay[a]	Coliphage	Limited screen	Routine assay[a]
1983								
2-16/2-17	x0			C	x0			x0
3-7/3-8	x			C	x			x
3-21/3-22	x0	x	x	G	x0	x	x	x0
4-4/4-5	x			C	x			x
4-18/4-19	x0	x	x	G	x0	x	x	x0
5-16/5-17								x0
6-27/6-28	x			C	x			x
7-11/7-12	x0	x		C	x0	x		x0
7-25/7-26	x			G	x			x
8-8/8-9	x0	x		G	x0	x		x0
8-22/8-23	x			G	x			x
9-12/9-13	x0			C	x0			x0
9-26/9-27								x0

x - composite water sample collected for indicated assay
0 - viral identification performed on this sample

a Total coliforms, fecal coliforms, fecal streptococci, total enteroviruses.
b C - composite sample; G - grab sample.

TABLE A-5. SUMMARY OF SAMPLING CONDITIONS--AEROSOL RUNS--OPERATIONAL YEAR 1982

	Sampled rig			Aerosol sampler location						Rig movement during run (m)	Mean wind direction θ_w[b]	Other rigs in operation		Wastewater	
					Line	Distance to rig, (m)									
Run no.	No.	Orientation	End gun status	Position/ tower	Angle θ_{sl}[a]	Single	Single	Pair	Pair			Possibly upwind	Not upwind	Source	Temp (°C)
M1	9	315°	On	Outer/6	35°	39	64	139	214	+2	25°	3	15	Pipeline	-
M2	2	130°	Off	Center/3	80°	35	60	135	210	-2	100°	None	3,5	Pipeline	-
M3	15	290°	Off	Center/4	60°	49	80	140	203	0	75°	None	3,5,8,11	Pipeline	-
M4	12	315°	On	Outer/5	30°	55	80	148	225	0	23°	2,7	6	Pipeline	-
M5	15	230°	Off	Outer/5	80°	64	115	174	288	0	113°	2,7		Pipeline	-
M6	3	50°	Off	Inner/3	45°	50	75	125	200	0	60°	12,19		Pipeline	-
M7	11	325°	Off	Outer/6	50°	61	87	155	236	0	60°	None	None	Reservoir	-
M8	15	70°	Off	Inner/3	75°	50	75	125	200	0	50°	None	None	Pipeline	-
M9	15	70°	Off	Inner/3	75°	55	80	130	205	0	90°	None	None	Reservoir	-
M10	4	330°	Off	Outer/6	70°	50	75	125	200	0	80°	None	None	Reservoir	-
M11	4	280°	Off	Center/4	85°	125	175	300	400	0	130°	None	None	Pipeline	27
M12	8	80°	Off	Center/4	90°	125	175	300	375	0	105°	None	None	Pipeline	-
M13	8	80°	Off	Center/4	90°	125	175	300	375	0	90°	None	None	Reservoir	-
M14	7	55°	Off	Center/3	75°	125	175	300	365	0	80°	4	6,11,12,13 17,19	Pipeline	27
M15	10	125°	Off	Center/4	65°	125	175	300	400	0	60°	6,7,8	11,13,17, 19,20[c]	Pipeline	-
M16	12	300°	On	Center/4	65°	50	75	125	200	0	65°	22	7,17,19, 20[c]	Reservoir	-
M17	14	30°	On	Center/4	90°	125	175	290	400	0	90°	7	2,4,8,9, 12,18,[c] 20,[c]21[c]	Pipeline	26
M18	14	20°	Off	Center/4	90°	125	175	275	400	0	85°	None	3,4,7,8, 9,11,12, 18,[c]20,[c] 21[c]	Pipeline	24
M19	9	90°	Off	Outer/5	65°	23	23	48	98	4	50°[d]	21[c]	2,8,11,15, 18,19	Reservoir	-
M20	10	130°	Off	Outer/5	85°	80	130	255	323	90	110°	6,7	15,18,19	Pipeline	28

a θ_{sl} - angle of sampler line with rig ($0° \leq \theta_{sl} \leq 90°$)

b θ_w - mean angle of wind with the rig during the run, measured in same direction from rig as θ_{sl}

c Rig with drops

d From Climatronics Weather Station at the tech plot

TABLE A-6. SAMPLER OPERATING VOLTAGE ON THE MICROORGANISM AEROSOL RUNS

Aerosol run number	Operating high voltage of large volume samplers (kV)														
	Upwind of irrigation rig		Downwind of irrigation nozzle line												
			20-39 m		40-59 m		60-89 m	90-149 m		150-249 m		250-349 m		350-409 m	
PREPLANTING IRRIGATION															
M1	?	?	14				14	12.2	13.8	14					
M2	13	11.5	11				11	12.8	12.8	14	15				
M3	12	9			10		6	12.5	12.5	10	12.5				
M4	14	14			11		10	12	12	12.5	12.5				
M5	13	13					12	9		12	12	12.5	13		
M6	12	14			10		11	12	13	12	12.5				
SUMMER CROP PIPELINE IRRIGATION															
M7	12.7	10.7			12		12	12	12	11	12				
M8	14	12			12		12	12.8	12.4	12	12				
M11	15	12.5						12		12		12	11	14	13
M12	12	12						12		12		12.8	12.5	13	12
M14	13	13.5						10		12		13.2	12.8	11.5	12
M15	13	13						12		12		12.5	13	12.8	11
M17	12	12						13		12.5		12.8	12.8	13	13
M18	12	12						14.5		16.5		12.8		14	13
M20	12	12						14		14		16.4	11	14	14
SUMMER CROP RESERVOIR IRRIGATION															
M9	15	12			12		12	11.5	12.4	12.7	12.7				
M10	13	13			12		12	12.8	12.5	12.5	12				
M13	12	11.5						12		11		12.5	12.8	12	13
M16	12.5	13.5			12		12	12.6	13.2	12.5	12.8				
M19	12	12	14	14.5	12	14.5		14	14						

? - Voltage not recorded.

TABLE A-7. SUMMARY OF SAMPLING CONDITIONS--QUALITY ASSURANCE RUNS--OPERATIONAL YEAR 1982

Run no.	Sampled rig			Aerosol sampler location		Rig movement during run (m)	Mean wind direction θ_w[a]	Other rigs in operation		Wastewater Source
	No.	Orien-tation	End gun status	Position/ tower	Distance to rig (m)			Possibly upwind	Not upwind	
Q1	11	340°	Off	Right/4-5	75	0	110°	None	None	Pipeline
Q2	15	65°	On	Center/3	50	0	75°	None	None	Pipeline

[a] θ_w - mean angle of wind with the rig during the run, measured in same direction from rig as θ_{sl}

TABLE A-8. SUMMARY OF SAMPLING CONDITIONS--VIRUS RUNS--OPERATIONAL YEAR 1982

Run No.	Segment No.	Sampled rig			Aerosol sampler location			Rig movement during segment (m)	Mean wind direction θ_w	Other rigs in operation		Wastewater Source
		No.	Orien-tation	End gun status	Position/ tower	Distance to rig (m) Start	Finish			Possibly upwind	Not upwind	
V1	1	4	320°	Off	Right/4-5	60	60	0	30°	None	None	Pipeline
	2	"	320°	"	"	60	60	0	50°	"	"	"
	3	"	325°	"	"	55	55	0	105°	"	"	"
	4	"	325°	"	"	55	55	0	110°	"	"	"
	5	"	325°	"	"	55	55	0	110°	"	"	"
V2	1	17	60°	Off	Center/5	50	47	3	105°	None	2,4,6,7, 11,12,13 17,19	Pipeline
	2	"	60°	"	"	45	42	3	110°	"	"	"
	3	"	58°	"	"	50	47	3	105°	"	"	"
	4	"	58°	"	"	45	42	3	110°	"	"	"
	5	"	56°	"	"	52	49	3	115°	"	"	"
V3	1	14	70°	Off	Center/4	47	44	3	80°	4,7	6,11,13, 17,20	Pipeline
	2	"	70°	"	"	44	41	3	85°	"	"	"
	3	"	68°	"	"	50	47	3	55°	"	"	"
	4	"	68°	"	"	47	44	3	80°	"	"	"
	5	"	66°	"	"	50	47	3	55°	"	"	"
V4	1	14	35°	On	Center/5	60	54	6	-	7	2,4,8,9, 12,18,20, 21	Pipeline (27°C)
	2	"	32°	"	"	51	45	6	45°	"	"	"
	3	"	30°	"	"	55	49	6	75°	"	"	"
	4	"	27°	"	"	46	40	6	60°	"	"	"
	5	"	25°	"	"	50	44	6	35°	"	"	"

TABLE A-9. SUMMARY OF SAMPLING CONDITIONS--DYE RUNS--OPERATIONAL YEAR 1982

	Sampled rig					Aerosol sampler location							
				Tower		Line angle	Distance to rig (m)				Mean wind direction	Wastewater	
Run No.	No.	Orien-tation	End gun status	Left position	Right position	θ_{sl}[a]	Left position		Right position		θ_w[b]	Source	Temp (°C)
D1	15	230°	Off	3	5	65°	25	75	25	75	80°	Pipeline	-
D2	4	330°	Off	6	4	70°	25	75	25	75	90°	Pipeline	-
D3	4	330°	Off	6	4	70°	25	75	25	75	80°	Pipeline	-
D4	15	65°	On	3	5	90°	40	80	40	80	90°	Pipeline	25.5

[a] θ_{sl} - angle of sampler line with rig ($0° \leq \theta_{sl} \leq 90°$)

[b] θ_w - mean angle of wind with the rig during the run, measured in same direction from rig as θ_{sl}

TABLE A-10. SUMMARY OF SAMPLING CONDITIONS--PARTICLE SIZE RUNS--OPERATIONAL YEAR 1982

Run no.	Sampled rig			Aerosol sampler location					Rig movement during run (m)	Mean wind direction θ_w[b]	Other rigs in operation		Wastewater Source
	No.	Orien-tation	End gun status	Position/ tower	Line Angle θ_{sl}[a]	Distance to rig, (m) Pair	Pair	Pair			Possibly upwind	Not upwind	
P1	2	130°	Off	Center/3	80°	36	61	86	0	70°	None	3,5	Pipeline
P2	11	330°	Off	Right/6	85°	33	58	83	0	30°	None	None	Pipeline
P3	15	70°	Off	Inner/3	75°	20	45	70	0	60°	None	None	Pipeline
P4	4	280°	Off	Center/4	85°	35	60	85	0	125°	None	None	Pipeline
P5	14	30°	Off	Center/5	60°	35	60	85	0	70°	None	3,4,7,8,9 11,12,18, 20,21	Pipeline

a θ_{sl} - angle of sampler line with rig ($0° \leq \theta_{sl} \leq 90°$)

b θ_w - mean angle of wind with the rig during the run, measured in same direction from rig as θ_{sl}

TABLE A-11. CORRECTION FACTOR FOR LVS OPERATING VOLTAGE (Referenced Basis of 12 kV)

Operating voltage (kV)	Correction factor (F)
6	0.33
8	0.36
9	0.38
10	0.42
11	0.47
11.5	0.80
12	1.00
12.5	1.15
13	1.25
13.5	1.32
14	1.33
14.5	1.32
15	1.29
16	1.24
17	1.22
18	1.21

TABLE A-12. SUMMARY OF METEOROLOGICAL CONDITIONS--AEROSOL RUNS--OPERATIONAL YEAR 1982

Run no. Run date Run time	Air temp (°C)		Mean wind direction (°)		Wind speed (m/sec)		Humidity at run location (%)	Dewpoint at EWS (°C)	Radiation at run location		
	At run location	EWS[a]	At run location (2 m)	EWS (10 m)	At run location (2 m)	EWS (10 m)			Cloud cover (8ths)	Cloud height	Solar radiation gcal/cm^2/min
M1/2-22-82 1850-1920	16	19.5	160	170	9.6	5.5	46	-12	3	NA	0
M2/2-23-82 1650-1720	26	29	200	205	6.7	7.0	50	-4	<1	-	0.73
M3/2-24-82 1400-1430	10	12.5	35	40	10.3	11.5	49	-17	7	High	0.90
M4/3-17-82 1535-1605	24	26.5	145	155	2.4	2.5	34	-5	6	High	0.93
M5/3-18-82 1230-1300	24	26	155	160	7.9	9.0	66	-7	4	High	0.95
M6/3-19-82 1148-1218	17	19	240	255	6.6	8.0	21	-12.5	<1	-	1.23
M7/7-7-82 1620-1650	29	30	85	110	11.4	NA	59	-4	4	Middle	0.51
M8/7-8-82 1353-1423	31	33	120	140	6.9	NA	54	-2	<1	-	1.25
M9/7-9-82 1331-1401	32	35	160	160	4.2	NA	29	-1	<1	-	1.26
M10/7-11-82 1530-1600	28	32	50	65	7.7	NA	51	-3	2	High	0.15
M11/7-14-82 1350-1420	31	32	150	180	6.4	NA	40	-2.5	2	High	1.35
M12/7-15-82 1114-1144	28	30	155	185	8.0	NA	51	-4	<1	-	1.10
M13/7-16-82 1025-1055	27	29	170	180	7.3	NA	54	-4.5	0	-	1.05
M14/8-3-82 1327-1357	33	33	155	210	4.3	NA	37	-1.5	0	-	1.20
M15/8-5-82 1211-1241	34	32	185	170	4.9	NA	24	-2.5	0	-	1.15
M16/8-6-82 1210-1240	31	33	235	240	3.3	NA	39	-1.5	0	-	1.17
M17/8-23-82 2030-2100	24	28	120	120	0.9	NA	59	-6	<1	-	After sunset
M18/8-25-82 2125-2155	22	25	115	140	0.6	1.25	77	-9	3	High	After sunset
M19/8-26-82 1422-1452	32	35	b	220	b	3.0	51	0	1	Middle & High	0.63
M20/8-27-82 1320-1350	35	35	200	235	2.5	2.5	31	0	<1	-	1.14

NA - not available

a Meteorological data collected from Climatronics Electronic Weather Station (EWS) at research plot

b Field met system malfunction

TABLE A-13. SUMMARY OF METEOROLOGICAL CONDITIONS--QUALITY ASSURANCE RUNS--OPERATIONAL YEAR 1982

Run no. Run date Run time	Air temp (°C)		Mean wind direction (°)		Wind speed (m/sec)		Humidity at run location (%)	Dewpoint at EWS (°C)	Radiation at run location		
	At run location	EWS[a]	At run location (2 m)	EWS (10 m)	At run location (2 m)	EWS (10 m)			Cloud cover (8ths)	Cloud height	Solar radiation gcal/cm2/min
Q1/3-15-82 1543-1613	19	11	230	250	9.4	11.5	30	-10.5	Blowing dust		0.44
Q2/7-13-82 1359-1429	29	30	170	190	3.8	NA	49	-4	<1	-	1.34

NA - not available

[a] Meteorological data collected from Climatronics Electronic Weather Station (EWS) at research plot.

TABLE A-14. SUMMARY OF METEOROLOGICAL CONDITIONS--VIRUS RUNS--OPERATIONAL YEAR 1982

Run no. Run date Run time	Segment no.	Air temp (°C)		Mean wind direction (°)		Wind speed (m/sec)		Humidity at run location (%)	Dewpoint at EWS (°C)	Radiation at run location		
		At run location	EWSa	At run location (2 m)	EWS (10 m)	At run location (2 m)	EWS (10 m)			Cloud cover (8ths)	Cloud height	Solar radiation gcal/cm2/min
V1/3-16-82												
1027-1057	1	14		290		6.0		41				0.93
1109-1139	2	-		270		3.5		-				1.12
1204-1234	3	17		215		4.6		42				1.20
1246-1316	4	19		210		4.5		40				1.12
1349-1419	5	22		210		5.8		27				1.14
	Avg	18	18	239	260	4.9	4.0	38	-13	6	High	1.10
V2/8-2-82												
1431-1501	1	31		155		4.8		51				1.24
1509-1539	2	31		155		5.2		51				1.15
1600-1630	3	31		150		5.1		40				1.05
1637-1707	4	31		150		5.0		42				0.95
1733-1803	5	31		155		5.9		40				0.69
	Avg	31	33.5	153	170	5.2	NA	45	-1	<1	High	1.02
V3/8-4-82												
1121-1151	1	29		150		4.5		53				1.08
1200-1230	2	30		155		4.7		50				1.15
1247-1317	3	32		125		3.4		43				1.20
1326-1356	4	32		150		2.9		52				1.15
1414-1444	5	33		125		4.0		40				1.18
	Avg	31	32	141	170	3.9	NA	48	-2.5	0	-	1.15
V4/8-24-82												
1113-1143	1	29		NA		NA		41				1.02
1153-1223	2	30		170		3.6		44				1.09
1246-1316	3	31		140		4.6		40				1.15
1326-1356	4	32		155		3.1		44				1.12
1426-1456	5	33		180		2.3		42				1.10
	Avg	31	33	161	180	3.4	NA	42	-2	0	-	1.10

NA - not available

a Meteorological data collected from Climatronics Electronic Weather Station (EWS) at research plot.

TABLE A-15. SUMMARY OF METEOROLOGICAL CONDITIONS--DYE RUNS--OPERATIONAL YEAR 1982

Run no. Run date Run time	Air temp (°C)		Mean wind direction (°)		Wind speed (m/sec)		Humidity at run location (%)	Dewpoint at EWS (°C)	Radiation at run location		
	At run location	EWS[a]	At run location (2 m)	EWS (10 m)	At run location (2 m)	EWS (10 m)			Cloud cover (8ths)	Cloud height	Solar radiation gcal/cm^2/min
D1/3-18-82 1455-1502	25	28	NA	160	NA	9.5	59	-6	4	High	0.55
D2/7-11-82 1733-1740	26	28.5	60	65	7.9	NA	63	-5	2	High	<0.05
D3/7-11-82 1752-1758	25	28	50	60	7.9	NA	63	-5.5	2	High	<0.05
D4/7-13-82 1533-1539	30	31.5	155	180	3.6	NA	50	-2	<1	-	1.34

NA - not available

[a] Meteorological data collected from Climatronics Electronic Weather Station (EWS) at research plot.

TABLE A-16. SUMMARY OF METEOROLOGICAL CONDITIONS--PARTICLE SIZE RUNS--OPERATIONAL YEAR 1982

Run no. Run date Run time	Air temp (°C)		Mean wind direction (°)		Wind speed (m/sec)		Humidity at run location (%)	Dewpoint at EWS (°C)	Radiation at run location		
	At run location	EWS[a]	At run location (2 m)	EWS (10 m)	At run location (2 m)	EWS (10 m)			Cloud cover (8ths)	Cloud height	Solar radiation gcal/cm^2/min
P1/2-23-82 1609-1619	28	29.5	200	210	7.8	7.2	20	-4	<1	-	0.73
P2/3-16-82 1539-1549	22	13.5	180	210	6.7	7.0	21	-8	6	High	0.61
P3/7-8-82 1510-1518	31	33.5	130	150	7.6	NA	46	-1.5	<1	-	1.21
P4/7-14-82 1519-1527	29	32.5	155	185	6.7	NA	43	-2	2	High	1.15
P5/8-25-82 1730-1738	29	31.5	100	120	2.5	NA	49	-1	5	High	NA

[a] Meteorological data collected from Climatronics Electronic Weather Station (EWS) at research plot.

TABLE A-17. RECOVERY OF SALMONELLA FROM WASTEWATER SAMPLES USING TWO PROCEDURES

	Standard selenite enrichment		Double enrichment[a]	
Sample	Salmonella detected	Volume enriched mL	Salmonella detected	Volume enriched mL
Lubbock-LV-7	-	200	+	100
Lubbock-LV-8	+	200	+	100
			+	10
			+	1
Lubbock-LV-9	+	200	+	10
			+	1
			+	0.1
Lubbock-LV-12	+	100	+	1
			+	0.1
			+	0.01
Lubbock-LV-13	-	100	+	0.1
			-	0.01
			-	1
Lubbock LV-14	+	25	+	0.1

[a] Kaper et al. (1977).

TABLE A-18. COMPARISON OF PROCEDURES FOR RECOVERY OF *YERSINIA ENTEROCOLITICA*--UNSEEDED SAMPLES

			Recovery of *Y. enterocolitica* from quadrant at plating time					
Enrichment	Medium	Treatment	Direct from sample	Zero	3 days	7 days	14 days	21 days
None	CAL		0[a]					
	CAL	KOH-NaCl	2					
	MAC		0					
	MAC	KOH-NaCl	0					
	SS		0					
	SS	KOH-NaCl	1					
0.067 M PBS	CAL			0	0	3	0	0
	CAL	KOH-NaCl		2	3	2	2	2
	MAC			0	0	0	0	0
	MAC	KOH-NaCl		2	2	3	3	3
	SS			0	0	0	0	0
	SS	KOH-NaCl		0	0	2	0	1
0.067 M PBS with 1% mannitol	CAL			0	0	0	0	0
	CAL	KOH-NaCl		2	2	2	3	3
	MAC			0	0	0	0	0
	MAC	KOH-NaCl		2	2	3	3	3
	SS			1	0	0	0	0
	SS	KOH-NaCl		0	0	2	2	3
0.85% NaCl with potassium tellurite (25 µg/mL)	CAL			0	2	0	0	0
	CAL	KOH-NaCl		0	0	0	0	0
	MAC			1	0	0	0	0
	MAC	KOH-NaCl		0	0	0	0	0
	SS			0	0	0	0	0
	SS	KOH-NaCl		0	0	0	0	0

[a] 0 = none detected

TABLE A-19. COMPARISON OF PROCEDURES FOR RECOVERY OF *YERSINIA ENTEROCOLITICA*--SEEDED SAMPLES

			Recovery of *Y. enterocolitica* from quadrant at plating time					
Enrichment	Medium	Treatment	Direct from sample	Zero	3 days	7 days	14 days	21 days
None	CAL		0[a]					
	CAL	KOH-NaCl	0					
	MAC		0					
	MAC	KOH-NaCl	0					
	SS		0					
	SS	KOH-NaCl	0					
0.067 M PBS	CAL			4	4	0	3	0
	CAL	KOH-NaCl		3	3	2	2	3
	MAC			3	0	4	0	0
	MAC	KOH-NaCl		3	2	3	3	2
	SS			2	0	3	0	0
	SS	KOH-NaCl		1	1	2	3	2
0.067 M PBS with 1% mannitol	CAL			3	3	3	0	0
	CAL	KOH-NaCl		3	2	3	4	3
	MAC			0	3	4	0	0
	MAC	KOH-NaCl		2	2	2	4	0
	SS			3	4	0	4	0
	SS	KOH-NaCl		2	1	1	3	2
0.85% NaCl with potassium tellurite (25 µg/mL)	CAL			3	0	0	0	0
	CAL	KOH-NaCl		0	0	0	0	0
	MAC			0	0	0	0	0
	MAC	KOH-NaCl		3	0	0	0	0
	SS			0	0	0	0	0
	SS	KOH-NaCl		0	0	0	0	0

[a] 0 = none detected

TABLE A-20. PARALLEL TESTING OF *CLOSTRIDRIUM PERFRINGENS* ASSAYS: COMPARISON OF MULTIPLE TUBE INOCULATION AND MEMBRANE FILTRATION TECHNIQUES

		Clostridrium perfringens enumerated by	
Sample	Heat treatment[a]	Multiple tube (MPN/100 mL)	Membrane filtration (cfu/100 mL)
Lubbock 4	+Δ	7.5×10^4	3.5×10^4
Wilson 4	+Δ	4.3×10^4	5.0×10^3
Lubbock 4	-Δ	2.1×10^6	5.0×10^4
Wilson 4	-Δ	7.5×10^4	1.5×10^4
Lubbock 5	+Δ	1.1×10^5	no growth[b]
Wilson 5	+Δ	2.4×10^4	no growth
Lubbock 5	-Δ	2.8×10^5	no growth
Wilson 5	-Δ	4.6×10^6	no growth
Lubbock 6	+Δ	1.5×10^4	5.9×10^4
Wilson 6	+Δ	2.1×10^4	6.9×10^4
Lubbock 6	-Δ	1.1×10^6	6.0×10^4
Wilson 6	-Δ	1.1×10^5	7.6×10^4

[a] Sample heated at 80°C for 30 minutes on multiple tube procedure and 65°C for 15 minutes on the membrane filtration procedure (Δ = heat).

[b] Increased volumes of sample tested were also negative for isolated colonies of *C. perfringens*.

TABLE A-21. VIRAL TYPES RECOVERED FROM WASTEWATER BY THE BENTONITE ADSORPTION PROCEDURE

Cell line	Viruses isolated
HeLa	Poliovirus 1, 2, 3
	Coxsackievirus A1, A7, A9, A10, A16
	Coxsackievirus B3, B4, B5
	Echovirus 1, 3, 6, 7, 11, 21, 25
BGM	Poliovirus 1, 2, 3
	Coxsackievirus B2, B3, B4, B5
	Echovirus 11, 25
RD	Poliovirus 2, 3
	Coxsackievirus B1
	Echovirus 6, 7, 11, 19, 22, 24, 30, 33

a Isolated from San Antonio, Lubbock, and Wilson samples; identified by a microneutralization technique using Lim Benyesh-Melnick typing pools.

TABLE A-22. VIRAL ISOLATES RECOVERED FROM THE SAME WASTEWATER SAMPLES BY VARIOUS ASSAY PROCEDURES

Sample	Type assay	Cell line	Viruses isolated[a]
Lubbock-1	Plaque	HeLa	Poliovirus 1, 2, 3 Coxsackievirus A1, A7, A9, A16 Coxsackievirus B3, B4, B5 Echovirus 1, 3, 6, 11, 21, 25
	Tube	BGM	Coxsackievirus B2, B3, B4, B5 Echovirus 11, 20, 24
	Tube	RD	Poliovirus 1 Coxsackievirus B1 Echovirus 6, 15, 24, 25, 29, 33
Lubbock-2	Plaque	HeLa	Poliovirus 2, 3 Coxsackievirus B2, B3, B5
	Tube	BGM	Coxsackievirus B2, B3, B5
	Tube	RD	Echovirus 11, 15, 19, 30
Wilson-1	Plaque	HeLa	Poliovirus 1, 3 Coxsackievirus A10 Echovirus 25
	Tube	BGM	Poliovirus 1 Coxsackievirus B2, B5 Echovirus 25
	Tube	RD	Poliovirus 3 Coxsackievirus B1 Echovirus 24

[a] Identified by a microneutralization technique using Lim Benyesh-Melnick typing pools.

TABLE A-23. ENTEROVIRUS ASSAY MATRIX FOR WASTEWATER SAMPLES

Cell line/assay system	Number of 100 mm plates/dilution Undiluted	10^{-1}
HeLa	10	10
HeLa + polio antisera	10	0
RD + polio antisera	10	5

TABLE A-24. VIRAL NEUTRALIZATION BY POLIOVIRUS ANTISERUM[a]

Antiserum Viral antigen	Batch Date	Test virus	Viral titer (pfu/mL) T_0	 $T_{30''}$	Neutralization ($T_{30''}/T_0$)
Type 1 polio (LSC)	6-63	Polio 1 (LSC)	1.6×10^4	$<5 \times 10^0$	$>3.1 \times 10^{-4}$
Type 2 polio (P-712)	7-65	Polio 2 (MEF)	1.8×10^5	5×10^0	2.8×10^{-5}
Type 3 polio (Leon)	6-65	Polio 3 (Sabin)	3.9×10^4	$<5 \times 10^0$	$>1.3 \times 10^{-4}$

[a] Results shown are for 1:100 dilution of rehydrated, heat-inactivated (56°C, 30 minutes) antiserum supplied as an NIH research reagent.

TABLE A-25. CONCENTRATION EFFICIENCY OF ORGANIC FLOCCULATION AND TWO-PHASE SEPARATION

Concentration procedure	% Polio 1[a] recovered	% CB3[a] recovered	% Echo 6[b] recovered
Organic flocculation			
0% beef extract	33	53	60
1% beef extract	41	61	79
2% beef extract	55	77	84
3% beef extract	33	62	81
Two-phase separation	50	61	43

[a] Results are an average of four experiments.

[b] Results are an average of two experiments.

TABLE A-26. LISS HEALTH DATA PROCESSING STATUS REPORT (excluding serology)

Data Collection Period	Start Date	Household Health Diary	Scheduled Fecal Bacteriology	Clinical Virology	Illness Specimen Bacteriology	Electron Microscopy	Activity Diary	Household/ Participant Interview	Polio Immunization
	1980								
011	May 18							ARKVP	
012	Jun. 1								
013	Jun. 15								
014	Jun. 29	ARKP							
015	Jul. 13	ARKP	ARCKVD	ARCKVD		AR			
016	Jul. 27	ARKP							
017	Aug. 10	ARKP	ARCKVD	ARCKVD		AR			
018	Aug. 24	ARKP							
019	Sept. 7	ARKP	ARCKVD	ARCKVD	ARKP	AR			
020	Sept. 21	ARKP							
	1981								
108	Apr. 5	LARKP	LARKVD	LARKVD		AR			ARKP
109	Apr. 19	LARKP							
110	May 3	LARKP	LARKVD	LARKVD		AR			ARKP
111	May 17	LARKP							
112	May 31	LARKP	LARKVD	LARKVD		AR			
113	Jun. 14	LARKP							ARKP
114	Jun. 28	LARKP	LARKVD	LARKVD	ARKP				
115	Jul. 12	LARKP							
116	Jul. 26								
117	Aug. 9	LARKP	LARKVD	LARKVD		AR			
118	Aug. 23	LARKP	LARKVD	LARKVD		AR			
119	Sept. 6	LARKP							
	1982								
201	Jan. 3	LARKV	LARKVD	LARKVD	ARKP	AR			ARKP
202	Jan. 17	LARKV			ARKP				
203	Jan. 31	LARKV			ARKP			ARKVP	
204	Feb. 14	LARKV							
205	Feb. 28	LARKV	LARKVD	LARKVD	ARKP	AR			
206	Mar. 14	LARKV			ARKP		LARCKVD		
207	Mar. 28	LARKV	LARKVD	LARKVD	ARKP	AR			
208	Apr. 11	LARKV					ARCKVD		
209	Apr. 25	LARKV							
210	May 9	LARKV							
211	May 23	LARKV							
212	Jun. 6	LARKV	LARKVD	LARKVD		AR			
213	Jun. 20	LARKV			ARKP				
214	Jul. 4	LARKV			ARKP				
215	Jul. 18	LARKV			ARKP				
216	Aug. 1	LARKV	LARKVD	LARKVD	ARKP	AR	LARCKVD		
217	Aug. 15	LARKV			ARKP				
218	Aug. 29	LARKV			ARKP				
219	Sept. 12	LARKV	LARKVD	LARKVD	ARKP	AR			
220	Sept. 26	LARKV			ARKP				
221	Oct. 10	LARKV			ARKP				
222	Oct. 24	LARKV			ARKP				
223	Nov. 7	LARKV			ARKP				
224	Nov. 21	LARKV			ARKP		ARCKVD		
225	Dec. 5	LARKV			ARKP				
226	Dec. 19								

continued...

TABLE A-26. (CONT'D)

Data Collection Period	Start Date	Household Health Diary	Scheduled Fecal Bacteriology	Clinical Virology	Illness Specimen Bacteriology	Electron Microscopy	Activity Diary	Household/ Participant Interview	Polio Immunization
	1983								
301	Jan. 2	LARKV			ARKP				
302	Jan. 16	LARKV			ARKP				
303	Jan. 30	LARKV	LARKVD	LARKP	ARKP	AR			
304	Feb. 13	LARKV			ARKP				
305	Feb. 27	LARKV			ARKP				
306	Mar. 13	LARKV							
307	Mar. 27	LARKV							
308	Apr. 10	LARKV	LARKVD	LARKP	ARKP	AR	ARCKVD		
309	Apr. 24	LARKV			ARKP				
310	May 8	LARKV			ARKP				
311	May 22	LARKV			ARKP				
312	Jun. 5	LARKV	LARKVD	LARKP	ARKP	AR			
313	Jun. 19	LARKV			ARKP				
314	Jul. 3	LARKV					ARCKVD		
315	Jul. 17	LARKV	LARKVD	LARKP	ARKP	AR			
316	Jul. 31	LARKV							
317	Aug. 14	LARKV	LARKVD	LARKP	ARKP	AR			
318	Aug. 28	LARKV			ARKP				
319	Sept. 11	LARKV							
320	Sept. 25	LARKV						ARKVP	
321	Oct. 9								
322	Oct. 23								
323	Nov. 6								
324	Nov. 20								
325	Dec. 4								
326	Dec. 18								

Status Codes

L - Labels Generated
S - Samples Stored
A - Activity Conducted
R - Received by Data Manager

C - Coded by Data Processing Group
K - Keypunched
P - Preliminary on Data Base
V - Verified
D - Data Processing Completed

TABLE A-27. SAMPLED MICROORGANISM DENSITIES ON THE QUALITY ASSURANCE AEROSOL RUNS

Quality assurance run number	Sampler alignment (from left to right)	Microorganism concentration in air			
		Fecal coliforms (cfu/m^3)	Fecal streptococci (cfu/m^3)	Mycobacteria (cfu/m^3)	Coliphage (pfu/m^3)
Q1[a,b]	(Wastewater conc., no./mL)	(51,000)	(4,800)	(20,000)	(1,100)
(75 m from nozzle line)	123	160,170,160		3.5	
	201	250	120,330,330		
	210		260		6.7,8.0,5.9
	211			2.6,5.3,5.3	11
	217	640	270	4.4	12
	219	TNTC,TNTC,TNTC		4.2	
	223	TNTC	280,210,280		
	226		390		8.3,10.4,8.3
	227			8.2,5.3,4.0	16
Q2	(Wastewater conc., no./mL)	(50,000)	(3,600)	(25,000)	(720[d])
(50 m from nozzle line)	210	87,90,80		≥0.60[c]	
	219	52	70,78,76		
	226		120		4.6,[d]3.8,[d]9.6[d]
	106			<0.15,<0.15,0.15	4.0[d]
	227	430	270	0.30	6.0[d]
	123	180,170,170		≥0.90[c]	
	211	200	53,60,50		
	223		38		7.0,[d]6.6,[d]7.2[d]
	217			2.0,0.52,0.67	10[d]

TNTC - too numerous to count

[a] Conducted during a dust storm.

[b] Portions received at laboratory at elevated temperature (9°C).

[c] A large number of colonies with indistinguishable morphology were present. Since only representative colonies were examined for acid fastness, reported data are minimal values.

[d] Possible laboratory contamination due to phage aerosolization.

TABLE A-28. CONSISTENCY OF AEROSOL MEASUREMENT PRECISION OVER DENSITY RANGE

Microorganism group/ sample set	Mean aerosol density (no./m^3)	Average coefficient of variation for replicate measurements
Fecal coliforms (cfu)		
Usual detection limit	0.1	
M1-M20: 6 pairs with $\bar{C}<1$	0.3	0.70
M1-M20: 8 pairs with $1<\bar{C}\leq 10$	3.3	0.60
M1-M20: 5 pairs with $\bar{C}>10$	21	0.37
Q2: 5 samplers	190	0.84
Q1: 3 samplers	350	0.82
AVERAGE OVER ALL SETS	0.3-350	0.67
Fecal streptococci (cfu)		
Usual detection limit	0.1	
M1-M20: 14 pairs with $\bar{C}<1$	0.3	0.71
M1-M20: 10 pairs with $1<\bar{C}<10$	3.7	0.52
M1-M20: 8 pairs with $1<\bar{C}<50$	27	0.21
M1-M20: 3 pairs with $\bar{C}>50$	75	0.20
Q2: 5 samplers	110	0.90
Q1: 5 samplers	290	0.21
AVERAGE OVER ALL SETS	0.3-290	0.46
Mycobacteria (cfu)		
Usual detection limit	0.1	
M1,M3-M16: 11 pairs with $\bar{C}<1$	0.3	0.75
Q2: 5 samplers	0.9	1.26
M1,M3-M16: 6 pairs with $\bar{C}\geq 1$	2.9	1.02
Q1: 5 samplers	4.5	0.20
AVERAGE OVER ALL SETS	0.3-4.5	0.81
Clostridium perfringens (cfu)		
Usual detection limit	0.3	
Sporulated: M17-M20: 3 pairs	0.8	0.69
Vegetative: M2,M17-M20: 7 pairs	1.6	0.74
AVERAGE OVER BOTH SETS		0.72
Coliphage (pfu)		
Usual detection limit	0.1	
M1-M20: 13 pairs with $\bar{C}<1$	0.3	0.56
M1-M20: 9 pairs with $1\leq\bar{C}\leq 5$	3.4	0.20
Q2: 5 samplers	6.7	0.37
Q1: 5 samplers	11.0	0.33
M1-M2: 3 pairs with $C>5$	11.1	0.71
AVERAGE OVER ALL SETS	0.3-11	0.43

TABLE A-29. ESTIMATED MAGNITUDE OF SOURCES OF PRECISION VARIATION

Microorganism group/ quality assurance run	Mean density in air (no./m^3)	Average coefficient of variation ($s/\bar{x}$)				
		Measurement variation (all sources)	Portion variation (shipping and lab sources)	Aliquot variation (lab sources)	Presumed shipping sources[a]	Presumed field sources[a]
Fecal coliforms (cfu)						
Q2	190	0.84	0.053	0.1	-[b]	0.8
Q1	350	0.82	0.040	0.06	-	0.8
Fecal streptococci (cfu)						
Q2	110	0.90	0.085	0.08	0.04	0.9
Q1	290	0.21	0.35	0.08	0.3	-
Mycobacteria (cfu)						
Q2	0.88	1.26	1.40	0.4	1.3	-
Q1	4.5	0.20	0.41			-
Coliphage (cfu)						
Q2	6.7	0.37	0.32	0.17	0.3	0.2
Q1	11.0	0.33	0.16	0.16	0	0.3

[a] Determined by subtraction of variances.

[b] Subtraction gives negative variance; presumably little variation due to this source.

TABLE A-30. POLIOVIRUS TITER REPRODUCIBILITY: COMPARISON OF RESULTS REPORTED BY U. OF ILLINOIS AND U. OF IOWA FOR THE SAME SERUM

Serum	Polio 1		Polio 2		Polio 3	
no.	Illinois	Iowa	Illinois	Iowa	Illinois	Iowa
1	>1024	256	32	64	8	32
2	32	16	16	8	4	<8
3	4	<8	<4	<8	<4	<8
4	32	16	128	64	16	8
5	32	64	256	512	8	8
6	64	128	64	128	8	8
7	16	16	16	64	8	16
8	8	8	256	256	8	16
9	8	16	16	32	<4	16
10	32	64	32	64	<4	8
11	16	16	32	>1024	32	128
12	8	8	16	64	<4	<8
13	4	<8	<4	<8	8	16
14	8	8	16	64	8	8
15	32	64	64	8	8	8
16	4	<8	4	128	8	16
17	16	128	32	64	64	64
18	256	256	32	128	8	8

TABLE A-31. REPEATABILITY OF CLINICAL BACTERIOLOGY RESULTS: SPLIT FECAL SPECIMENS

ID number	Reported results	QA results	Agreement[a]
Period 108			
55713	E. coli[b]	E. coli[b] C. albicans (VL)	+
55913	E. coli (M)	E. coli (H) E. cloacae (VL)	+
32111	E. coli (M)	E. coli (H)	+
43414	E. coli (M) K. oxytoca (L)	E. coli (M) K. oxytoca (L)	++
21112	E. coli (H) S. aureus (L) C. freundii H_2S^+ (L) C. freundii H_2S^- (VL)	E. coli (H) S. aureus (L) C. freundii (L) K. pneumoniae (VL)	+
53313	E. coli (H) K. oxytoca (L) H. alvei (L) E. cloacae (L) C. albicans (L)	E. coli (H) K. oxytoca (L) H. alvei (L) E. cloacae (L) C. albicans (L)	++
32412	S. aureus (H) K. pneumoniae (VL) K. pneumoniae[a] E. sakazakii[a]	S. aureus (H) K. pneumoniae (VL) K. pneumoniae[a] E. sakazakii[a]	++
12311	E. coli (H) S. aureus (L) K. pneumoniae (VL) E. cloacae (VL)	E. coli (H) S. aureus (L) K. pneumoniae (VL) E. cloacae (VL)	++
12302	E. coli (M) K. pneumoniae (VL)	E. coli (M) C. albicans (VL)	+
31011[c]	E. coli (M) C. albicans (VL)	E. coli (M) C. albicans (VL)	++
		E. coli (M) C. albicans (VL)	++
42613	E. coli (M)	E. coli (M)	++
22712	E. coli (M) K. oxytoca (VL) Fl. pseudomonas (VL)	E. coli (M)	+
53913	E. coli (H) S. aureus (L)	E. coli (H) S. aureus (L)	++

continued...

TABLE A-31. (CONT'D)

ID number	Reported results	QA results	Agreement
12202	E. coli (M) K. oxytoca (VL) C. freundii (VL)	E. coli (M) K. oxytoca (VL) C. freundii (VL) C. albicans (VL)	+
40812	E. coli (M) Fl. pseudomonas (VL)	E. coli (M) Fl. pseudomonas (VL)	++
Period 110			
55912	E. coli (M) S. aureus (L)	E. coli (M) S. aureus (L) E. cloacae (VL)	+
55913	E. coli (M) K. pneumoniae (VL) S. epidermidis (VL)	E. coli (M) K. pneumoniae (VL) S. epidermidis (VL)	++
42613	E. coli (M) K. oxytoca (L)	E. coli (M) K. oxytoca (L)	++
40411	E. coli (H)	E. coli (H)	++
12211	E. coli (H) C. albicans (M)	E. coli (H) C. albicans (M)	++
53312	E. coli (H) K. pneumoniae (L) C. albicans (VL)	E. coli (H) K. pneumoniae (L) C. albicans (VL)	++

a Degree of agreement:

- ++ Total agreement (same organisms identified and same level of growth) on split specimens.
- + The level of growth differed by one quadrant, or organisms were identified in one specimen at the VL level (1 to 10 colonies on plate) but not in the respective split specimen. Because of the small numbers of organisms represented by the VL level of growth, such differences are probably not significant.
- - Disagreement in identification of one or more organisms isolated at the light or greater level, or a two quadrant or greater discrepancy in level of growth.

b Isolated by enrichment procedures, therefore nonquantitative.

c Sample split into three portions, rather than two.

TABLE A-32. ACCURACY OF CLINICAL BACTERIOLOGY RESULTS: ANALYSIS OF SEEDED UNKNOWN FECAL SPECIMENS

Specimen	Identification reported	Level[a]	Correct identification	Level
1	Klebsiella pneumoniae	H	Klebsiella pneumoniae	H
	Shigella flexneri	H	Shigella flexneri	H
	Yersinia enterocolitica	H	Yersinia enterocolitica	H
2	Enterobacter cloacae	H	Enterobacter cloacae	H
	Salmonella species	H	Salmonella typhimurium	H
	Serratia marcescens	H	Serratia marcescens	H
	Staphylococcus aureus	H	Staphylococcus aureus	H
3	Klebsiella pneumoniae	H	Klebsiella pneumoniae	H
	Shigella flexneri	H	Shigella flexneri	H
	Yersinia enterocolitica	H	Yersinia enterocolitica	H
4	Candida albicans	H	Candida albicans	H
	Escherichia coli	H	Escherichia coli	H
	Proteus vulgaris	H	Proteus vulgaris	H

[a] Quantitation of growth: H - heavy.

TABLE A-33. LEVELS OF GROWTH REPRESENTED BY DIFFERENT CONCENTRATIONS OF KNOWN ORGANISMS: FECAL SPECIMEN PROCEDURE

Organism	Seeded organism concentration, cfu/mL	Level of quantitation from clinical lab report[a]	
Escherichia coli	9×10^1	NG	VL
	9×10^2	L	L
	9×10^3	L	L
	9×10^4	L	L
	4.5×10^6	M	L
	9×10^6	M	L
	4.5×10^7	M	M
	9×10^7	M	H
Klebsiella pneumoniae	0	NG	NG
	33	VL	NG
	3.3×10^3	NG	NG
	3.3×10^5	L	M
	3.3×10^6	M	H
Pseudomonas aeruginosa	7	NG	NG
	700	NG	NG
	7.0×10^4	L	L
	7.0×10^6	M	M
	7.0×10^7	H	H

[a] Quantitation of growth on duplicate platings of seeded unknowns:

NG - negative	M - moderate
VL - very light	H - heavy
L - light	

TABLE A-34. LEVELS OF GROWTH REPRESENTED BY DIFFERENT CONCENTRATIONS OF KNOWN ORGANISMS: THROAT SWAB PROCEDURE

Organism	Seeded organism concentration, cfu/mL	Level of quantitation from clinical lab report[a]	
Escherichia coli	4.0×10^{2}	L	VL
	4.0×10^{3}	L	L
	4.0×10^{4}	M	L
	4.0×10^{5}	M	M
	4.0×10^{6}	H	M
	4.0×10^{7}	H	H
	4.0×10^{8}	H	H
Enterobacter cloacae	3.1×10^{2}	VL	VL
	3.1×10^{3}	L	L
	3.1×10^{4}	M	L
	3.1×10^{5}	M	M
	3.1×10^{6}	M	M
	3.1×10^{7}	H	M
	3.1×10^{8}	H	H
Klebsiella pneumoniae	2.7×10^{2}	VL	VL
	2.7×10^{3}	L	L
	2.7×10^{4}	M	M
	2.7×10^{5}	M	M
	2.7×10^{6}	H	M
	2.7×10^{7}	H	H
	2.7×10^{8}	H	H
Streptococcus pyogenes	1.8×10^{1}	NG	NG
	1.8×10^{2}	VL	VL
	1.8×10^{3}	L	L
	1.8×10^{4}	M	M
	1.8×10^{5}	M	L
	1.8×10^{6}	H	M
	1.8×10^{7}	H	H

[a] Quantitation of growth on duplicate platings of seeded unknowns:

NG - negative
VL - very light
L - light
M - moderate
H - heavy

TABLE A-35. REPEATABILITY OF CLINICAL VIROLOGY RESULTS: SPLIT FECAL SPECIMENS

Period	Sample	Participant	Routine Analysis[a]		QA Analysis[a]	
			HeLa	RD	HeLa	RD
116	1	22712[b]	0/2	0/2	0/2	0/2
	2	22712[b]			0/2	2/2
	3	12211	0/2	0/2	0/2	0/2
	4	12202	0/2	0/2	0/2	0/2
	5	10413	0/2	0/2	0/2	0/2
	6	55912	0/2	0/2	0/2	0/2
	7	55913	0/2	0/2	0/2	0/2
	8	32412	0/2	0/2	0/2	0/2
	9	23112	0/2	0/2	0/2	0/2
117 and 118	1	32411	0/2	0/2	0/2	0/2
	2	53912	0/2	0/2	0/2	0/2
	3	53911[b]	1/2	0/2	0/2	1/2
	4	53911[b]			0/2	0/2
	5	20211	0/2	0/2	0/2	0/2
	6	53313	1/2	0/2	2/2	0/2
	7	22512	0/2	0/2	0/2	0/2
	8	40311	0/2	0/2	0/2	0/2
	9	40312	0/2	0/2	0/2	0/2
	10	56211	0/2	0/2	0/2	0/2
	11	56202	0/2	0/2	0/2	0/2
	12	45114	0/2	0/2	0/2	0/2
	13	53312	0/2	0/2	0/2	0/2
	14	45113	0/2	0/2	0/2	0/2
	15	40312[b]	0/2	0/2	0/2	0/2
	18	40312[b]			0/2	0/2
	16	40311[b]	0/2	0/2	0/2	0/2
	17	40311[b]			0/2	0/2
	19	40216	0/2	0/2	0/2	1/2
	20	12202	0/2	0/2	0/2	0/2
	21	12211	0/2	0/2	0/2	0/2
	22	55715	0/2	0/2	0/2	1/2
	23	32412	0/2	0/2	0/2	0/2
	25	53911	0/2	0/2	0/2	0/2
	26	55911	0/2	0/2	0/2	0/2
	27	22512	0/2	0/2	0/2	0/2
	28	40214	0/2	0/2	0/2	0/2
	29	43613	0/2	0/2	0/2	0/2

[a] Number of tubes showing viral cpe/total number of tubes inoculated for each cell line listed. Other cells used with negative results were BGM and primary RhMK.

[b] Replicate QA sample.

TABLE A-36. ENVIRONMENTAL QUALITY ASSURANCE: REPLICATE ANALYSES OF SPLIT WASTEWATER SAMPLES

Analysis	Source	Sample 1	Sample 2	Mean
Bacteriology[a]				
Fecal coliform	Wilson LV-9	4.2×10^6/100 mL	3.9×10^6/100 mL	4.1×10^6
	Lubbock LV-9	8.8×10^6/100 mL	8.4×10^6/100 mL	8.6×10^6
	Wilson LV-10	6.9×10^7/100 mL	6.2×10^7/100 mL	6.6×10^7
Total coliform	Lubbock LV-9	1.5×10^7/100 mL	1.6×10^7/100 mL	1.6×10^7
Fecal streptococci	Lubbock LV-9	4.2×10^5/100 mL	4.8×10^5/100 mL	4.5×10^5
Virology				
Enteroviruses on HeLa (unaltered)	Lubbock LV-9	1.1×10^2 pfu/L	1.2×10^2 pfu/L	1.2×10^2
	Wilson LV-10	1.6×10^2 pfu/L	1.7×10^2 pfu/L	1.7×10^2

a Membrane filtration.

TABLE A-37. REPRODUCIBILITY IN SEPARATE LABORATORIES OF BACTERIAL INDICATOR DENSITIES IN WASTEWATER DURING BASELINE PERIOD

Sample date	Total coliform (cfu/100 mL)		Fecal coliform (cfu/100 mL)	
	LCCIWR	UTSA	LCCIWR	UTSA
6-4-80	4.3×10^7	3.5×10^7	Not done	8.7×10^6
7-29-80	5.0×10^7	3.8×10^7	2.5×10^7	7.2×10^6
11-4-80	3.2×10^7	1.4×10^7	1.5×10^7	8.8×10^6
1-20-81	1.0×10^7	6.0×10^6	2.0×10^6	1.5×10^6
2-17-81	1.5×10^7	1.1×10^7	4.6×10^6	3.4×10^6
3-10-81	2.7×10^7	1.2×10^7	4.5×10^6	1.6×10^6
3-24-81	1.8×10^7	1.6×10^7	4.0×10^6	8.3×10^6
4-21-81	4.0×10^7	5.2×10^7	5.3×10^6	5.9×10^6
5-5-81	2.9×10^7	Not done	5.9×10^6	8.6×10^6

TABLE A-38. REPRODUCIBILITY IN SEPARATE LABORATORIES OF FECAL COLIFORM DENSITIES IN WASTEWATER DURING 1982 AND 1983

	Fecal coliforms (colonies/mL)					
	Hancock reservoir		Pipeline effluent		Wilson Imhoff influent	
Sampling date	UTSA[a]	LCCIWR	UTSA[a]	LCCIWR	UTA	LCCIWR
2-15/16-82			39	30		
2-15/16-82[b]			11,000	97,000		
3-1/2-82			5,600	30,000		
3-8/9-82			75,000	100,000		
3-15/16-82			79,000	180,000		
3-22/23-82			81,000	50,000		
3-29/30-82			55,000	52,000		
4-5/6-82			84,000	16,000		
4-19/20-82			110,000			
4-26/27-82			9,100			
6-14/15-82	520	940 (600)[c]	66,000	55,000		
6-29/30-82	60	200	68,000	60,000		
7-26/27-82	190		58,000			
8-9/10-82	390	370	35,000	20,000 (30,000)		
8-30/31-82	10	2 (1.7)	200	41		
9-13/14-82	350	700 (490)	65,000	34,000		
	UTA		UTA		UTA	
11-1/2-82	3.5	2.8	49,000	90,000	130,000[d]	90,000
12-13/14-82	730	180	31,000	40,000	110,000[d]	100,000
2-16/17-83	15	10	59,000	4,000	14,000	40,000
3-7/8-83	4	1.7	23,000	18,000	150,000	180,000
3-21/22-83	150	90	6,100	20,000	76,000	45,000 (60,000)
4-4/5-83	100	44	20,000	14,000	150,000	51,000
4-18/19-83	440	200	18,000	10,000	130,000	90,000
5-16/17-83	-	-	-	-	350,000	60,000
6-27/28-83	300	160	59,000	39,000	260,000	54,000
7-11/12-83	150	5.5	53,000	27,000	370,000	180,000
7-25/26-83	3	1	48,000	40,000	240,000	13,000
8-8/9-83	110	50	120,000	40,000	310,000	90,000
8-22/23-83	30	1.7	90,000	20	230,000	20,000

a mean of triplicate assays
b trickling filter plant effluent
c parenthetical value, when given, is the result of a duplicate analysis
d samples taken as Imhoff tank effluent

TABLE A-39. REPRODUCIBILITY IN SEPARATE LABORATORIES OF FECAL STREPTOCOCCI DENSITIES IN WASTEWATER DURING 1982 AND 1983

	Fecal streptococci (colonies/mL)			
	Hancock reservoir		Pipeline effluent	
Sampling date	UTSA[a]	LCCIWR[b]	UTSA[a]	LCCIWR[b]
2-15/16-82			120	40
3-1/2-82			1,000	400
3-8/9-82			5,900	5,000
3-15/16-82			3,500	4,000
3-22/23-82			7,900	2,200
3-29/30-82			5,000	2,600
4-5/6-82			2,800	1,400
4-19/20-82			4,800	
4-26/27-82			1,800	
6-14/15-82	20	12.8	1,000	1,890 (1,500)
6-29/30-82	3	10	4,200	1,800
7-26/27-82	3		2,300	
8-9/10-82	6.6	6.0	2,500	1,000 (2,000)
8-30/31-82	0.3	1.1	30	61
9-13/14-82	10	100 (20)	3,500	5,100

a Mean of triplicate assays.

b Parenthetical value, when given, is the result of a duplicate analysis.

TABLE A-40. IDENTIFICATION OF FECAL COLIFORM ISOLATES

Sample		Fecal coliform isolates				Nonfecal coliform isolates			
Source	Date	Oxidase −		Oxidase +		Oxidase −		Oxidase +	
Hancock reservoir	7-25/26-83		8		0		ND		ND
		E. cloacae	2						
		E. coli	2						
		K. pneumoniae	3						
		Unidentified[a]	1						
		TOTAL ID	8						
Wilson influent	7-25/26-83		23		0		ND		ND
		E. coli	13						
		K. oxytoca	2						
		K. pneumoniae	6						
		Unidentified	2						
		TOTAL ID	23						
Wilson influent	8-8/9-83		27		3		10		5
		E. coli	4	A. hydrophila	2	E. aerogenes	1	Fl. Pseudomonas	1
		Klebsiella sp.	3	TOTAL ID	2	H. alvei	1	NSC[b]	3
		Citrobacter sp.	1			Klebsiella sp.	3	TOTAL ID	4
		Total ID	8			Total ID	7		

a Based on carbohydrate utilization, probably Klebsiella sp., but retesting necessary for positive ID.

b No such code, presumably not common member of Enterobacteriaceae family.

TABLE A-41. REPRODUCIBILITY OF TOTAL ORGANIC CARBON RESULTS FOR WASTEWATER DURING 1982 AND 1983

	TOC (mg/L)			
	Hancock reservoir		Pipeline effluent	
Sampling date	UTA	LCCIWR	UTA	LCCIWR
11-1/2-82	28	27	54	41
2-16/17-83	19	19	49	48
3-7/8-83	28	25	109	67
3-21/22-83	23	15	83	75
4-4/5-83	26	21	62	52
4-18/19-83	34	23	50	47
6-27/28-83	17	13	42	36
7-11/12-83	21	17	35	26
7-25/26-83	24	21	22	34
8-8/9-83	27	29	28	32
8-22/23-83	33	49	32	26

APPENDIX B

INITIAL PERSONAL INTERVIEW QUESTIONNAIRE

HH name ______________________
____
HH # ______________________
Phone # ______________________
HH size ______________________

University of Illinois
School of Public Health

Lubbock Land Treatment Project:

Personal Interview for Health Watch

(*Time Interview Began* __________ *am*/*pm*)

ASSURANCE OF CONFIDENTIALITY - All information that would permit identification of individuals will be held in strict confidence, will be used only by persons engaged in and for the purpose of the survey and will not be disclosed or released to others for any purpose. The results will be used only when combined with those of many other people.

First, I would like to ask you a few questions about your household.

1. a. Do you have air conditioning in your home?

Yes	1
No *(Skip to Q. 2)*	0

b. Do you have

central air conditioning or	1
window or wall units or	2
both	3

c. During the summer, do you have the air conditioning on:

all or most of the time	1
some of the time every day	2
only when it is very hot or	3
never	4

2. Do you obtain your drinking water from

a private well, or	1
public water supply	2

3. Do you dispose of sewage through

a septic tank or cespool or	1
city sewage system	2

Now, I would like to ask you some questions about household members and their activities.

4. a. Including yourself, how many people live in this household? _______

b. How many of these people are related to you? ______________________

If there are unrelated household members (HM):

I will be asking you some questions about each of your family members. I will be talking with unrelated household members separately.

5. a. Beginning with yourself, please tell me the first name of each person now living in the household who is related to you.

b. How is __________________________ related to you? *(Record relationship and sex)*

6. In what year (were you/was ______) born?

7. a. Do you (does ______) have a job or go to school outside your home or farm?

Yes	1
No *(Skip to Q. 8)*	0

b. Looking at the map, please show me where (you/_____) works or goes to school. *(Indicate Zone)*

Zone	1
Zone	2
Lubbock	3
Other area (excluding Lubbock)	4

8. Approximately how many hours per week (do you/does _____) spend outside the outlined area shown on this map? *(Show map)*

9. During the non-winter months, how many hours per day (do you/does _____) generally spend out of doors, within the outlined area on: *(Show map)*

a. Weekdays

less than 1 hour/day	1
more than 1; less than 4 hrs./day	2
more than 4; less than 8 hrs./day	3
more than 8 hrs./day	4

b. Weekends

less than 1 hr./day	1
more than 1; less than 4 hrs./day	2
more than 4; less than 8 hrs./day	3
more than 8 hrs./day	4

Ask Questions 10 through 14 if household is located on a farm. Skip to Q. 15 if household is not located on a farm.

10. How many hours per week (do you/does _____) spend doing farm work out of doors?

0	1
less than 10 hrs./week	2
more than 10; less than 20 hrs./week	3
more than 20; less than 40 hrs./week	4
more than 40 hrs./week	5

We also need to find out a little bit about your farm, so we can more accurately judge what types of farm work household members might be doing.

11. What crops are you producing on your farm this year? Please tell me each crop which you are growing, and the amount of acreage devoted to it. *(Check as many as apply)*

Crop		Acreage
None	____	None (000)
cotton	____	____
wheat	____	____
other	____	____

12. What types of livestock are you raising on your farm this year? Please tell me each type of livestock and the number of animals. *(Check as many as apply)*

Livestock		Number
None	____	None (000)
cattle	____	____
hogs	____	____
sheep	____	____
fowl	____	____
other	____	____

13. a. Do you currently irrigate your farm land?

Yes	1
No *(Skip to Q. 14)*	0

b. What is the source of that water?

Well	1
Other (specify)	2

14. Approximately how many acres of land do you farm, including pastures, fallow ground and grazing land? ____________________

Skip to Q. 16

Ask Q. 15 if household is not located on a farm.

15. a. Do you or does anyone in your household ever work on a farm within the outlined area? *(show map)*

Yes 1
No *(Skip to Q. 16)* 0

b. Who is that?

c. How many weeks per year (do you/does _____) work on a farm?

d. How many days per week (do you/does _____) work on a farm, when (you/_____) work(s)?

e. During which season(s) (do you/does _____) generally work on a farm? *(Check as many as apply)*

a. Spring
b. Summer
c. Fall
d. Winter

16. a. Approximately how many times per month (do you/does _____) travel to Lubbock?

b. Approximately how much time (do you/does _____) spend in Lubbock on each visit?

17. a. Do you or does anyone in your household drink bottled water regularly?

Yes 1
No *(Skip to Q. 18)* 0

b. Who is that?

c. Do you/does _____ ever drink water from the tap?

Yes 1
No 0

Now I would like to find out about any long term or chronic illnesses or conditions which you or anyone in this household has ever had which required consultation with a doctor.

18. a. Have you or anyone in this household ever seen a doctor for any of these respiratory illnesses or conditions? *(Show card A)*

Yes		1
No	*(Skip to Q. 19)*	0
DK	*(Skip to Q. 19)*	8

b. Who is that?

For each yes to Q. 18a ask:

c. Which illness or conditions (do you/does ____) have? *(Check as many as apply)*

a. Allergies
b. Chronic bronchitis
c. Emphysema
d. Asthma
e. Tumor of cancer of the lung
f. Tumor of cancer of the mouth or throat
g. Other (specify)

d. How old (were you/was _____) when the ____________________ *(read condition)* first appeared?

(For each illness circled, record age on adjacent line)

e. What medications and/or treatments, if any, (are you/is _____) taking for (your/his/her) ____________________ *(read condition)*?

19. a. Have you or has anyone in this household ever seen a doctor for any of these heart conditions? *(Show card B)*

Yes	1
No *(Skip to Q. 20)*	0
DK *(Skip to Q. 20)*	8

b. Who is that?

For each yes to Q. 19a ask:

c. Which type of heart condition (do you/does _____) have?

- a. High blood pressure
- b. Stroke
- c. Heart attack
- d. Angina
- e. Other (specify)

d. How old (were you/was _____) when the ________________ *(read condition)* first occurred?

e. What medications and/or treatments, if any, (are you/is _____) taking for (your/her/his) ________________________ *(read condition)*?

20. a. Have you or has anyone in this household ever seen a doctor for any of these stomach or abdominal conditions? *(Show card C)*

Yes		1
No	*(Skip to Q. 21)*	0
DK	*(Skip to Q. 21)*	8

b. Who is that?

For each yes to Q. 20a ask:

c. What of these conditions (do you/does _____ have? Tumor or cancer of the

a. Stomach
b. Intestine
c. Colon
d. Esophagus
e. Stomach (peptic) or intestinal (duodenal) ulcer
f. Ulcer of the colon (ulcerative colitis)
g. Diverticulosis
h. Gall bladder problems
i. Other (specify)

d. How old (were you/was_____) when the ____________________ *(read condition)* first appeared?

e. What medications and/or treatments, if any, (are you/is _____) currently taking for (your/her/his) ________________ *(read condition)*?

21. a. Have you or has anyone in this household ever seen a doctor for any of these other types of conditions? *(Show card D)*

Yes		1
No	*(Skip to Q. 23)*	0
DK	*(Skip to Q. 23)*	8

b. Who is that?

For each yes to Q. 21a ask:

c. Which of these conditions (do you/does____) have?

a. Skin cancer
b. Leukemia
c. Hodgkin's Disease
d. Other cancers
e. Arthritis
f. Diabetes
g. Anemia
h. Immunological disorder
i. Rheumatic fever
j. Serum hepatitis (Hepatitis B)
k. Infectious Hepatitis (Hepatitis A)
l. Infectious mononucleosis
m. Other chronic conditions

d. How old (were you/was _____) when the ____________________ *(read condition)* first appeared?

e. What medications and/or treatments, if any, (are you/is _____) currently taking for the ______________________ *(read condition)*?

---

22. *Specified medication/treatments*

23. a. Have you or has anyone in this household ever had a blood transfusion?

Yes		1
No	*(Skip to Q. 24)*	0
DK	*(Skip to Q. 24)*	8

b. Who is that?

24. a. Have you or has anyone in this household ever been on a kidney machine or hemodialysis?

Yes		1
No	*(Skip to Q. 25)*	0
DK	*(Skip to Q. 25)*	8

b. Who is that?

25. a. Have you or has anyone in the household ever been in close contact with (i.e. lived with or helped care for) a person who had TB (tuberculosis)?

Yes		1
No	*(Skip to Q. 26)*	0
DK	*(Skip to Q. 26)*	8

b. Who is that?

26. a. Do you or does anyone in this household smoke cigarettes regularly?

Yes		1
No	*(Skip to Q. 27)*	0
DK	*(Skip to Q. 27)*	8

b. Who is that?

For each HM born before 1962, ask Q. 27 thru Q. 29

27. Are you (is _____) currently working at any part-time or full-time job? *(exclude housewifery)*

Yes	1
No *(Skip to Q. 29)*	0

If HM is *not* *currently working, ask:*

28. Are you (is_____): *(Read categories)*

Usually employed, but just out of work temporarily		1
Retired		2
Homemaker	*(Skip to Q. 30)*	3
Disabled or handicapped	*(Skip to Q. 30)*	4
Not usually employed	*(Skip to Q. 30)*	5
Student	*(Skip to Q. 30)*	6
Other (Specify)	*(Skip to Q. 30)*	7

29. a. What (is/was) your/_____'s) main occupation or job title?

b. What kind of work (do/did) you/_____) do? That is, what (are/were) (your/_____'s) duties on the job?

(If occupation is not "farmer", ask Q. 29c)

c. What (does/did) (your/_____'s) employer manufacture or sell, or what services does it provide?

Ask Q. 30 only for respondent, and if applicable, respondent's spouse

30. What is the highest grade of school which (you/_____) (have/has) completed?

None	0
Elementary	1 2 3 4 5 6 7 8
High School	9 10 11 12
College	13 14 15 16
Some graduate or professional school	17
Graduate or professional degree	18

31. Which household members contribute to the financial support of this household? ______________________________

32. a. Considering all of the income from employment, net farm income and from all other sources, please tell me which category on this card best describe your total household income before taxes in 1979? *(Show card E)*

a.	less than 5,000	1
b.	5,000 - 7,999	2
c.	8,000 - 9,999	3
d.	10,000 - 14,999	4
e.	15,000 - 19,999	5
f.	20,000 - 29,999	6
g.	30,000 and over	7
h.	*DK (ask 32B)*	8
i.	*Refused (ask 32B)*	9

b. Can you tell me if it was:

less than 10,000 or	1
more than 10,000	2
DK	8
Refused	9

33. Now, in case the office finds I've missed something what would be the best time to call you? ______________________ a.m.
p.m.

****

I	II	III	IV	V	VI
____	____	____	____	____	____
(Respondent)	____	____	____	____	____
Male......1 *Female....2*	*Male......1* *Female....2*	*Male......1* *Female....2*	*Male......1* *Female....2*	*Male......1* *Female....2*	*Male......1* *Female....2*
19 _ _ _ _	19 _ _ _ _	19 _ _ _ _	19 _ _ _ _	19 _ _ _ _	19 _ _ _ _
(Age ____)	(Age ____)	(Age ____)	(Age ____)	(Age ____)	(Age ____)

34. *Please Record Phone # on front of questionnaire*

35. *Record race of respondent*

White/Caucasian	*1*
Black/Negro	*2*
Oriental/Asian	*3*
Latino/Mexican/Puerto Rican	*4*
*Other(Specify)*______________	*5*

37. *Does the respondent live in a:*

Single family dwelling	*1*
Building for 2 families or duplex	*2*
Apartment house (3-4 units)	*3*
Apartment house (5 or more units)	*4*

38. *Is the household located on a farm?*

Yes	*1*
No	*2*

Time interview ended ______ *a.m.*
p.m.

APPENDIX C

PERSONAL QUESTIONNAIRE UPDATE IN FEBRUARY 1982

House Card only.

HH # 01 ________________________ 1-5

Name ________________________ 6-20

Phone # ________________________ 21-27

HH Size ________________________ 28-29

Interviewer ________________________

University of Illinois
School of Public Health

Lubbock Health Effects Study
Personal Interview Update

(Date of Interview________)

ASSURANCE OF CONFIDENTIALITY - All information that would permit identification of individuals will be held in strict confidence, will be used only by persons engaged in and for the purpose of the survey and will not be disclosed or released to others for any purpose. The results will be used only when combined with those of many other people.

HOUSEHOLD INFORMATION

1a. Have you changed residences since you enrolled in the Health Watch ?

Yes *(Skip to Q. 2)*	**1**
No	**2**

b. Have you made any of the following changes in your residence since you enrolled in the Health Watch?

a. Installed air conditioning *(Ask 2b-c)*
b. Changed water supplies *(Ask 3a)*
c. Changed waste disposal *(Ask 3b)*

			Card Columns
2a. Do you now have air conditioning in your home?			
	Yes	1	30
	No *(Skip to Q. 3)*	2	
b. Do you have central air conditioning or		1	31
	window or wall units	2	
	or both	3	
c. During the summer, do you have the air conditioning on:			
	All or most of the time	1	32
	Some of the time everyday	2	
	Only when it is very hot	3	
	Never	4	
3a. Do you now obtain your drinking water from			
	A private well, or	1	33
	public water supply	2	
b. Do you now dispose of sewage through:			
	A septic tank or cesspool	1	34
	or city sewage system	2	

End of Household File

PARTICIPANT INFORMATION

1a **Has anyone left your household permanently or temporarily since you enrolled in the Health Watch?**

Yes	1
No *(Skip to Q. 2)*	2

b. **Who was that?**

For each "yes" to Q. 1a-b, ask the following questions.

c. **When did ______ leave?** *(Record month, year)*

d. **Did _______ leave permanently?**

Yes *(Skip to Q. 2)*	1
No	2

e. **When did ______ return?** *(Record month, year. If HM has not returned record "NR" and ask 1f.)*

f. **When do you expect ______ to return?** *(Record month, year. Record "DK" if return not known.)*

2a. **Have you added any new members, including infants, to your household since you enrolled in the Health Watch?**

Yes	1
No *(Skip to Q. 3)*	2

*b. **What is his or her name?** *(Record name in column at top of facing page.)*

For each new household member, ask the following questions:

*c. **How is ________ related to you?** *(Record in column at top of facing page.)*

*d. **What is _______'s sex?** *(Record in column at top of facing page.)*

*e. **What is _______'s age?** *(Record in column at top of facing page.)*

f. **When did _________ enter your household?** *(Record month, year.)*

g. **How long will _______ be staying with you?** *(Record "permanently" for infants and other permanent residents. Otherwise record length of stay in weeks.)*

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best available copy.

Now, I would like to ask you about any long-term or chronic illnesses which you or anyone in your household may have developed since you enrolled in the Health Watch. If you are not sure whether a household member developed a condition before or after enrolling in the study, please tell me about it anyway and we can check that later.

3a. Have you or has anyone in your household been newly diagnosed as having any of these respiratory illnesses or conditions since you enrolled in the study?

Read list of conditions. Pause after each condition to allow respondent to reply. For each "yes", ask "Who was that?" *and record condition in appropriate column.*

a. Allergies
b. Chronic bronchitis
c. Emphysema
d. Asthma
e. Tumor or cancer of the lung
f. Tumor or cancer of the mouth or throat
g. Other *(specify)*

Ask 3b. for each condition reported.

b. What medications and/or treatments, if any, (are you/is ______) taking for the ____________? *(Record medications.)*
(read condition)

4a. Have you or has anyone in your household been newly diagnosed as having any of these cardiovascular conditions since you enrolled in the study?

Read list of conditions. Pause after each condition to allow respondent to reply. For each "yes", ask "Who was that?" *and record condition in appropriate column.*

a. High blood pressure
b. Stroke
c. Heart attack
d. Angina
e. Other *(specify)*

Ask 4b. for each condition reported.

b. What medications and/or treatments, if any, (are you/is _______) taking for the ________________? *(Record medications.)*
(read condition)

5a. Have you or has anyone in your household been newly diagnosed as having any of these stomach or abdominal conditions since you enrolled in the study?

Read list of conditions. Pause after each conditions to allow respondent to reply. For each "yes", ask "Who is that? *and record condition in appropriate column.*

Tumor or cancer of the:

a. Stomach
b. Intestine
c. Colon
d. Esophagus

e. Stomach (peptic) or intestinal (duodenal) ulcer
f. Ulcer of the colon (ulcerative colitis)
g. Diverticulosis
h. Gall bladder problems
i. Other *(specify)*

Ask 5b. for each condition reported.

b. What medications and/or treatments, if any, (are you/is _____) taking for the ______________? *(Record all medications.)*

6a. Have you or has anyone in your household been newly diagnosed as having any of these other types of conditions since you enrolled in the study?

Read list of conditions. Pause after each condition to allow respondent to reply. For each "yes", ask "Who is that?" *and record condition in appropriate column.*

a. Skin cancer
b. Leukemia
c. Hodgkin's Disease
d. Other cancers
e. Arthritis
f. Diabetes
g. Anemia
h. Immunological disorder
i. Rheumatic fever
j. Serum hepatitis (hepatitis B)
k. Infectious hepatitis (hepatitis A)
l. Infectious mononucleosis
m. Other chronic conditions *(specify)*

Ask 6b. for each condition reported.

b. What medications and/or treatments, if any, (are you/is_______) taking for the _____________? *(Record all medications.)*

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7a. Have you or has anyone in your household started working, stopped working or changed jobs since you enrolled in the study?

Yes	1
No *(Skip to Q. 8)*	2

b. Who was that?

Stopped working *(Skip to Q. 8)*	1
Started working	2
Changed jobs	3

c. What is the name of the place where (you/______) now work(s)? *(Record place)*

d. What is (your/ _______'s) new job title? *(Record job title)*

Now, I would like to ask you about a couple of other types of health conditions which are of interest to us. We want to know if you or anyone in your household has ever seen a doctor for these conditions.

8a. Have you or has anyone in your household ever seen a doctor for a goiter or other thyroid condition?

Yes	1
No *(Skip to Q. 9)*	2
DK *(Skip to Q. 9)*	8

b. Who is that?

c. Please tell me what the doctor called the thyroid condition, if you know. *(Record condition if known. Enter "DK" if not known.)*

d. How old (were you/was_________) when the thyroid condition first occurred? *(Record age.)*

e. Do you/does _______ __ still have the thyroid condition?

Yes	1
No	2

f. What medications or treatments have you/has _________ ever received for the thyroid condition? *(Record all medications and treatments.)*

g. Which of those medications or treatments, if any, (are you/ is _________) currently taking for the thyroid condition? *(Record all current medications and treatments.)*

9a, Have you or has anyone in your household ever seen a doctor for pneumonia?

Yes	1
No *(Skip to Q. 10, if applicable)*	2
DK *(Skip to Q. 10, if applicable)*	8

b. Who is that? *(Record condition in appropriate column.)*

c. How many times have you/has _ ______ _ had pneumonia? *(Record # times.)*

d. How old (were you/was______) the last time that the pneumonia occurred? *(Record age.)*

e. Were you/was_ _____ ever hospitalized for pneumonia?

Yes	1
No	2
DK	8

f. Approximately how long did the pneumonia last the last time that it occurred? *(Record duration in weeks.)*

This question is to be asked only for children 18 years of age or less. Ask appropriate questions for age of each child.

10a. Where (did/does _____) go to grammar school? *(Record all schools attended and location of school.)*

b. Where (did/does_____) to to junior high or middle school? *(Record all schools attended and location of school.)*

c. Where (did/does _____) go to high school? *(Record all schools attended and location of school.)*

d. Did ______ ever receive a polio immunization at school?

Yes	1
No *(End of interview)*	2
DK *(End of interview)*	8

e. Could you please tell me which school that was? *(Record name and location of school.)*

APPENDIX D

PERSONAL QUESTIONNAIRE UPDATE IN OCTOBER 1983

HH# ______________________________

Name ______________________________

Phone # ______________________________

Current HH Size ______________________________

Interviewer ______________________________

Date of Interview ______________________________

University of Illinois
School of Public Health

LUBBOCK HEALTH EFFECTS STUDY
1983 PERSONAL QUESTIONNAIRE UPDATE

QUEST3

ASSURANCE OF CONFIDENTIALITY -- All information that would permit identification of individuals will be held in strict confidence, will be used only by persons engaged in and for the purpose of the survey and will not be disclosed or released to others for any purpose. The results will be used only when combined with those of many other people

Household File

1. Has your household moved since January 1982?

   When did you move? (Record month and year)

   Where are you now living? (Record approximate location)

2. a. Do you have air conditioning in your home?

YES	1	ACOND
NO (Skip of Q. 3)	0	

   b. Do you have:

Central air conditioning - refrigeration	1	
Central air conditioning - evaporative cooler	2	ACNAME
Window or wall units -- refrigeration	3	
Window or wall units -- evaporative cooler	4	

   c. During the summer, do you have the air conditioning on:

All or most of the time	1	
Some of the time every day	2	ACUSE
Only when it is very hot	3	
Never	4	

3. a. Do you obtain your drinking water from:

A private well (go to b.)	1	DWATER
Public water supply (go to d.)	2	

   b. Do you chlorinate your well water?

YES	1		WCHLOR
NO	2	(Go to Q. 4.)	

c. How frequently is chlorine added to your water? (Choose best answer)

Continually (automatic chlorinator)	1	
Daily	2	
Weekly	3	
Monthly	4	FCHLOR
Only when well is known to be contaminated	5	

(GO TO Q. 3)

d. Is your water supplied by:

City of Wilson	1	
Canadian River	2	PWATER

4. Do you dispose of sewage through:

A septic tank or cesspool	1	
City sewage system	2	SEWAGE

5. What is the highest level of education achieved by any member of the household? (Include children who have left home)

None	0								
Elementary	1	2	3	4	5	6	7	8	
High school	9	10	11	12					HEDUC
College	13	14	15	16					
Some graduate or professional school	17								
Graduate or professional degree	18								

ASK THE FOLLOWING QUESTIONS FOR HOUSEHOLDS THAT FARM:

6. Approximately how many acres of land do members of your household farm? (Include fallow ground, pastures and grazing land)

____________ Acres ACRES3

7. What crops are you producing on your farm this year? Please tell me the amount of acreage and if any acreage usually used for that crop is fallow due to the payment in kind program.

CROP	ACRES PLANTED	PAYMENT IN KIND ACREAGE	
Cotton	______	______	COTTON3
Wheat	______	______	WHEAT3
Oats	______	______	OATS3
Milo	______	______	MILO3
______	______	______	OTHER3
______	______	______	
	APLANT3	APIK3	

8. What types of livestock are you raising this year?

LIVESTOCK	NUMBER	
Cattle	____	CATTLE3
Hogs	____	HOGS3
Sheep	____	SHEEP3
Fowl	____	FOWL3
______	____	OTHERL3
______	____	

9. Do you currently irrigate your farmland?

YES 1
NO 2

IRRIG3

10. What is the source of that water and approximately how many acres are irrigated by that source?

	# of acres	
Well	______	IWELL
Wastewater	______	IWASTE

Participant information

1. Enter Participant ID, Name and birthdate on opposite page.

2. a. Has anyone left your household permanently or temporarily since January 1982?

YES	1
NO (Skip to Q. 3.)	0

b. Who was that? (Record names)

(FOR EACH "YES" TO Q. 2 a.-b., ASK THE FOLLOWING QUESTION)

c. When did __________ leave? (Record month, year)

Now I would like to ask you about any longterm or chronic illnesses which you or anyone in your household may have developed since January 1982. If you are not sure whether a household member developed a condition before or after January 1982, please tell me about it anyway and we can check that later.

3. a. Have you or has anyone in your household been newly diagnosed as having any of these respiratory illnesses or conditions since January 1982?

(READ LIST OF CONDITIONS. PAUSE AFTER EACH CONDITION TO ALLOW RESPONDENT TO REPLY. FOR EACH "YES", ASK "WHO WAS THAT?" AND RECORD CONDITION IN APPROPRIATE COLUMN.)

a. Allergies
b. Chronic bronchitis
c. Emphysema
d. Asthma RESP
e. Tumor or cancer of the lung
f. Tumor or cancer of the mouth or throat
g. Other (specify)

(ASK 3.b. FOR EACH CONDITION REPORTED)

b. What medications and/or treatments, if any, are you/is _______ taking for the ________________? (RECORD MEDICATIONS)
(read condition)

4. a. Have you or has anyone in your household been newly diagnosed as having any of these cardiovascular conditions since January 1982?

(READ LIST OF CONDITIONS. PAUSE AFTER EACH CONDITION TO ALLOW RESPONDENT TO REPLY. FOR EACH "YES", ASK "WHO WAS THAT?" AND RECORD CONDITION IN APPROPRIATE COLUMN.)

a. High blood pressure
b. Stroke
c. Heart attack
d. Angina
e. Other (specify)

HEART

(ASK 4.b. FOR EACH CONDITION REPORTED)

b. What medications and/or treatment, if any, are you/is ________ taking for the ____________? (read condition) (RECORD MEDICATIONS)

5. a. Have you or has anyone in your household been newly diagnosed as having any of these stomach or abdominal conditions since January 1982?

(READ LIST OF CONDITIONS. PAUSE AFTER EACH CONDITION TO ALLOW RESPONDENT TO REPLY. FOR EACH "YES", ASK "WHO IS THAT?" AND RECORD CONDITION IN APPROPRIATE COLUMN.)

Tumor or cancer of the:
a. Stomach
b. Intestine
c. Colon
d. Esophagus
e. Stomach (peptic) or intestinal (duodenal) ulcer
f. Ulcer of the colon (ulcerative colitis)
g. Diverticulosis
h. Gall bladder problems
i. Other (specify)

ABDOM

(ASK 5.b. FOR EACH CONDITION REPORTED)

B. What medications and/or treatments, if any, are you/is ________ taking for the ____________? (read condition) (RECORD MEDICATIONS)

6. a. Have you or has anyone in your household been newly diagnosed as having any of these other types of conditions since January 1982?

(READ LIST OF CONDITIONS. PAUSE AFTER EACH CONDITION TO ALLOW RESPONDENT TO REPLY. FOR EACH "YES", ASK "WHO IS THAT?" AND RECORD CONDITION IN APPROPRIATE COLUMN.)

a. Skin cancer
b. Leukemia
c. Hodgkin's Disease
d. Other cancers
e. Arthritis
f. Diabetes OTHERO
g. Anemia
h. Immunological disorder
i. Rheumatic fever
j. Serum hepatitis (Hepatitis B)
k. Infectious hepatitis (Hepatitis A)
l. Infectious mononucleosis
m. Other chronic conditions (specify)

(ASK 6.b. FOR EACH CONDITION REPORTED)

b. What medications and/or treatments, if any, are you/is ________ taking for the ____________?
(read condition)

(RECORD ALL MEDICATIONS)

7. a. Did you or anyone in your household see a doctor for a goiter or other thyroid condition during 1982 or 1983?

NO (Skip to Q. 8)	0
YES	1

b. Who is that?

c. Please tell me what the doctor called the thyroid condition if you know.

(RECORD CONDITION IF KNOWN. ENTER "DK" IF NOT KNOWN.)

d. What medications or treatments have you/has _______ ever received for the thyroid condition?

(RECORD ALL MEDICATIONS AND TREATMENTS.)

8. a. Did you or anyone in your household see a doctor for pneumonia during 1982 or 1983?

NO	0	
YES	1	PNEU
DK	8	

b. Who is that? (RECORD CONDITION IN APPROPRIATE COLUMN)

c. How old were you/ was ________ at the time that the pneumonia occurred? (RECORD AGE) PNEUAGE

d. Were you/was ________ hospitalized?

NO	0	
YES	1	PNEUHOS
DK	8	

e. Approximately how long did the pneumonia last the last time that it occurred? (RECORD DURATION IN WEEKS.) PNEUDUR

9. a. Do you or does anyone in your household drink bottled water regularly?

YES	1	BOTTLED3
NO (Skip to Q. 10)	0	

b. Who is that?

c. Do you/does ________ ever drink water from the tap?

YES	1	TAP WATER3
NO (Skip to Q. 11)	0	

10. Compared to other people in your/________'s age group, how much tap water do you/________ drink? (Include beverages made with tapwater, i.e. coffee, tea, Kool-Aid.)

Less than average	1	
Average	2	WCONSM
More than average	3	

11. a. Do you or does anyone in this household smoke cigarettes regularly?

YES	1	
NO	2	SMOKE3
DK	3	

b. Who is that?

c. How much do you/does _______ smoke in a day?

One half pack or less per day	1	
One half to one pack per day	2	
More than one pack per day	3	PACKDAY

12. a. Does anyone in this household chew tobacco on a regular basis?

NO	0	
YES	1	TCHEW
DK	8	

b. Who is that?

13. a. Have you or has anyone in your household started working, stopped working or changed jobs since January 1982?

NO	0	
YES	1	WORKS3

b. Who was that? (RECORD NAME AND STATUS)

Stopped working (also ask 13. c.)	1
Started working (also ask 13. d.)	2
Changed jobs (also ask 13. d.)	3

c. Are you/ is _______ : (READ CATEGORIES)

Usually employed, but just out of work temporarily	1	
Retired	2	
Homemaker (Skip to Q. 14)	3	EMPSTAT3
Disabled or handicapped (Skip to Q. 14)	4	
Not usually employed (Skip to Q. 14)	5	
Student (Skip to Q. 14)	6	
Other (specify)	7	

d. What is the name of the place where you/ _________ now work(s)?

(RECORD PLACE)

What is your/ _________'s new job title?

(RECORD JOB TITLE)

OCCUP3

14. How many occasions a week do you/does __________ have contact with large groups of people (large = 10 or more people)?

Less than once a week	1
One to 5 times a week	2
6 - 10 times a week	3
11 - 15 times a week	4
15 or more times	5

CONTACT

(INCLUDES SCHOOL ATTENDENCE, CHURCH MEETINGS, SOCIAL OCCASIONS, CONGREGATIONS AT THE COTTON GIN, ETC.).

15. Does your family, or your spouse's family, have a history of cancer?

YES	1
NO	0

HCANCER

Would you mind giving us some information about these relatives? (Include spouse, if deceased, children, grandparents, siblings and aunts or uncles)

NAME	RELATIONSHIP (to respondent or respondent's spouse)	TYPE OF CANCER	YEAR (DIAGNOSED OR DIED)	# YEARS LIVED IN LYNN COUNTY (if none, enter 0)
NCANCER				LYNNCO

APPENDIX E

INFORMED AND PARENTAL CONSENT FORMS

ADULT'S CONSENT FOR PARTICIPATION IN A HEALTH RESEARCH PROJECT

I, ____________________ , state that I am _______ years of age and
(NAME OF PARTICIPANT)
wish to participate in an infectious disease study being conducted by the School of Public Health at the University of Illinois under the direction of Dr. Robert L. Northrop and Southwest Research Institute Project Manager, David Camann.

The purpose of the research is to ascertain the number and types of infections and other illnesses I will have, during most of 1983, to evaluate the health effects, if any, of aerosols emitted from nearby irrigation rigs spraying wastewater.

This project involves my allowing you to obtain from me two (2) blood samples and tuberculin tests in 1983.

I understand that there are no experimental procedures to be performed on me in this research and that there are no personal risks involved.

I understand that there may be a mild and temporary sensation in the skin when the blood sample and skin tests are done and that bleeding at these sites is not expected if I follow instructions of the professional staff performing these procedures.

I acknowledge that I have been informed that this research is designed to assist in maintaining or improving my personal health and will benefit me personally if causes for my infections are found.

I understand that in the event of physical injury resulting from this research there is no compensation and/or payment for medical treatment from the University of Illinois at the Health Sciences Center or Southwest Research Institute for such injury except as may be required of the University or Institute by law.

I acknowledge that Doctor Northrop, Mr. Camann or their representative explained to me the need for the research; has informed me that I may withdraw from participation at any time and has offered to answer any inquiries which I may make concerning the procedures to be followed.

I freely and voluntarily consent to my participation in this research project.

If you have any questions, we expect you to ask us. If you have any additional questions later, Dr. Northrop (312 - 996-4759: call collect. P.O. Box 6998, Chicago Il. 60680) will be happy to answer them.

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE. YOUR SIGNATURE INDICATES THAT YOU HAVE DECIDED TO PARTICIPATE HAVING READ THE INFORMATION PROVIDED ABOVE.

____
Date

____
Signature of Witness to explanation
(not signature)

____
Signature

MINOR'S CONSENT FOR PARTICIPATION
IN A HEALTH RESEARCH PROJECT
FORM CM

I, ____________________ , state that I am _______years of age and
(NAME OF PARTICIPANT)
wish to participate in an infectious disease study being conducted by the School of Public Health at the University of Illinois under the direction of Dr. Robert L. Northrop and Southwest Research Institute Project Manager, David Camann.

The purpose of the research is to ascertain the number and types of infections and other illnesses I will have, during most of 1983, to evaluate the health effects, if any, of aerosols emitted from nearby irrigation rigs spraying wastewater.

This project involves my allowing you to obtain from me two (2) blood samples and tuberculin tests in 1983.

I understand that there are no experimental procedures to be performed on me in this research and that there are no personal risks involved.

I understand that there may be a mild and temporary sensation in the skin when the blood sample and skin tests are done and that bleeding at these sites is not expected if I follow instructions of the professional staff performing these procedures.

I acknowledge that I have been informed that this research is designed to assist in maintaining or improving my personal health and will benefit me personally if causes for my infections are found.

I understand that in the event of physical injury resulting from this research there is no compensation and/or payment for medical treatment from the University of Illinois at the Health Sciences Center or Southwest Research Institute for such injury except as may be required of the University or Institute by law.

I acknowledge that Doctor Northrop, Mr. Camann or their representative explained to me the need for the research; has informed me that I may withdraw from participation at any time and has offered to answer any inquiries which I may make concerning the procedures to be followed.

I freely and voluntarily consent to my participation in this research project.

If you have any questions, we expect you to ask us. If you have any additional questions later, Dr. Northrop (312 - 996-4759: call collect; P.O. Box 6998, Chicago Il. 60680) will be happy to answer them.

YOU ARE MAKING A DECISION WHETHER OR NOT TO PARTICIPATE. YOUR SIGNATURE INDICATES THAT YOU HAVE DECIDED TO PARTICIPATE HAVING READ THE INFORMATION PROVIDED ABOVE.

____
Date

Reproduced from best available copy.

____
Signature

Minor's Name
(Please Print)

PARENTAL CONSENT

We, parents or guardians of the above minor volunteer, agree to the participation of the above minor in the research project set out above. We have been informed of the need for the research, the benefits to be derived from it, and the risks involved. We have been informed that the research cannot be conducted with adults only because of the nature of the research.

We also understand that in the event of physical injury resulting from this research there is no compensation and/or payment for medical treatment from the University of Illinois at the Medical Center or Southwest Research Institute for such injury except as may be required of the University or Institute by law.

Being aware of the necessity for the participation of minors in this research project and being informed that the procedures will also benefit the above-named minor personally by reporting to me/us, the parents or guardians, and to his or her physician, test results which may assist in diagnosis of an infectious illness the minor may have during this study, we consent to the minor's participation.

(SIGNATURE OF PARENTS OR GUARDIANS)

(SIGNATURE OF PARENTS OF GUARDIANS)

(WITNESS TO EXPLANATION)
(NOT TO SIGNATURE)

(DATE)

IMPORTANT INFORMATION
ABOUT POLIO AND INACTIVATED POLIO VACCINE
Please read this carefully

IP 10/1/80

WHAT IS POLIO? Polio is a virus disease that often causes permanent crippling (paralysis). One person out of every 10 who get polio disease dies from it. There used to be thousands of cases and hundreds of deaths from polio every year in the United States. Since polio vaccine became available in the mid 1950's, polio has nearly been eliminated. In the last five years, fewer than 25 cases have been reported each year. It's hard to say exactly what the risk is of getting polio at the present. Even for someone who is not vaccinated, the risk is very low. However, if we do not keep our children protected by vaccination the risk of polio will go back up again.

INACTIVATED POLIO VACCINE (IPV): Immunization with inactivated polio vaccine is effective in preventing polio and has successfully controlled polio in several countries. The vaccine is given by injection. Several doses are needed to provide good protection. Young children should get three doses in the first year of life, each separated by 1 to 2 months, and another dose 6 to 12 months later, at about 18 months of age. A booster dose is needed every 3 to 5 years, especially when children enter school or when there is a high risk of polio, for example, during an epidemic or when traveling to a place where polio is common. The vaccine is effective in providing protection to over 90% of people who receive it.

POSSIBLE SIDE EFFECTS FROM THE VACCINE: Inactivated polio vaccine is not known to produce any side effects.

PREGNANCY: Polio vaccine experts do not think inactivated polio vaccine can cause special problems for pregnant women on their unborn babies. However, doctors usually avoid giving any drugs or vaccines to pregnant women unless there is a specific need. Pregnant women should check with a doctor before taking inactivated polio vaccine.

WARNING — SOME PERSONS SHOULD *NOT* TAKE INACTIVATED POLIO VACCINE WITHOUT CHECKING WITH A DOCTOR:

- Those who are sick right now with something more serious than a cold.
- Those with allergies to antibiotics called neomycin or streptomycin
- Pregnant women

NOTE ON ORAL POLIO VACCINE: Besides the inactivated polio vaccine, there is also an oral polio vaccine which is given by mouth and which after several doses protects against polio for a long time, probably for life. Many polio experts feel that the oral vaccine is more effective for preventing the spread of polio and for controlling polio in the United States. However, it should not be given to persons who have a low resistance to infection or who live with persons with low resistance to infections. It has been associated very rarely with paralysis in persons who receive the vaccine or who are in close contact with those recently vaccinated. Oral polio vaccine is widely used in this country. It can be given alone or in combination with IPV. If you would like to know more about oral polio vaccine or combinations of oral and inactivated vaccine, please ask us.

QUESTIONS: If you have any questions about polio or polio vaccination, please ask us now or call your doctor or health department before you sign this form.

REACTIONS: If the person who received the vaccine gets sick and visits a doctor, hospital, or clinic in the 4 weeks after vaccination, please report it to:

TEXAS DEPARTMENT OF HEALTH
NURSING DIVISION 797-4331

PLEASE KEEP THIS PART OF THE INFORMATION SHEET FOR YOUR RECORDS

I have read the information on this form about polio and the inactivated vaccine. I have had a chance to ask questions which were answered to my satisfaction. I believe I understand the benefits and risks of inactivated polio vaccine and request that it be given to me or to the person named below for whom I am authorized to make this request.

IP 10/1/80

INFORMATION ON PERSON TO RECEIVE VACCINE
(Please print first three lines)

Name (last) (first) (middle) Birthdate Age
Address County
City State Zip Code
X
Signature of person to receive vaccine or person authorized to make the request Date

FOR CLINIC USE

Clinic Ident.
Date Vaccinated
Manufacturer and Lot No.
Site of administration

TEXAS DEPARTMENT OF HEALTH

INFORMACION IMPORTANTE ACERCA DE LA POLIOMIELITIS Y LA VACUNA ANTIPOLIO ATENUADA

Favor de leer cuidadosamente

IP 10/1/80

¿QUE ES LA POLIOMIELITIS? La poliomielitis (polio) es una enfermedad causada por un virus y que muchas veces resulta en parálisis permanente. Muere aproximadamente 1 de cada 10 personas que se contagian de ella. Antes ocurían miles de casos de polio y centenares de muertes causadas por esta enfermedad todos los años en los Estados Unidos. Desde que se hizo disponible la vacuna antipolio a mediados de la década de los cincuentas. la poliomielitis ha sido casi totalmente eliminada. En los últimos 5 años. se han reportado menos de 25 casos en cada año. Es difícil señalar con exactitud el riesgo actual de contagiarse de polio. Aun para las personas no vacunadas. el riesgo es muy reducido. Sin embargo. si no mantenemos la protección de nuestros hijos por medio de la vacunación regular. el riesgo de contraer polio volverá a aumentar.

LA VACUNA ANTIPOLIO ATENUADA (IPV): La inmunización por medio de la vacuna antipolio atenuada sirve efectivamente para prevenir la poliomielitis. y ha logrado controlar la enfermedad en varios países. La vacuna se administra en forma de inyección. Se requieren varias dosis para lograr una protección satisfactoria. Los bebés deben recibir 3 dosis en su primer año de vida. con una separación de 1 o 2 meses entre cada dosis. y deben recibir otra dosis entre 6 y 12 meses después. a los 18 meses de edad aproximadamente. Se requiere una dosis de refuerzo cada 3 o 5 años. particularmente cuando los niños entren a la escuela o cuando haya un alto riesgo de contraer polio. como por ejemplo durante una epidemia. o durante viajes a lugares donde la poliomielitis es una enfermedad común. La vacuna protege eficazmente a más del 90% de las personas que la reciben.

EFECTOS SECUNDARIOS DE LA VACUNA: Por lo que se sepa. la vacuna antipolio atenuada no produce efecto secundario alguno.

MUJERES EMBARAZADAS: Los expertos en vacunas antipolio no creen que la vacuna antipolio atenuada cause problemas para mujeres embarazadas. ni para sus niños aún no nacidos. Sin embargo. los médicos generalmente se abstienen de recetar drogas o vacunas para mujeres embarazadas. a menos que haya alguna necesidad específica de ello. Las mujeres embarazadas deben consultar con un médico antes de tomar la vacuna antipolio atenuada.

PRECAUCION — ALGUNAS PERSONAS NO DEBEN RECIBIR LA VACUNA ANTIPOLIO ATENUADA SIN CONSULTAR PRIMERO CON UN MEDICO:

- — Las personas que sufren actualmente de cualquiera enfermedad mś seria que un catarro.
- — Las personas que padezcan alergias a los antibióticos conocidos como Neomicina y Estreptomicina.
- — Las mujeres embarazadas.

NOTA SOBRE LA VACUNA ANTIPOLIO DE ADMINISTRACION ORAL: Además de la vacuna antipolio atenuada. existe también una vacuna antipolio de administración oral. que se toma por la boca. y que. despueś de varias dosis. ofrece protección contra la poliomielitis por un tiempo largo. probablemente por toda la vida. Algunos expertos creen que la vacuna oral es más eficaz para prevenir la propagación de polio y para controlar esta enfermedad en los Estados Unidos. Sin embargo. la vacuna oral no se debe administrar a personas que tengan una baja resistencia a infecciones. ni a las que vivan con otras personas que tengan una baja resistencia a infecciones. En ciertas ocasiones raras. esta vacuna ha sido asociada con la parálisis en personas que han recibido la vacuna o que han estado en contacto íntimo con otras personas recién vacunadas. La vacuna antipolio oral se usa ampliamente en este país. Puede ser administrada sola o junto con la IPV (vacuna antipolio atenuada). Si usted desea saber más acerca de la vacuna antipolio oral. o acerca de las combinaciones de vacuna atenuada y oral. por favor consúltenos.

PREGUNTAS: Si tiene usted alguna pregunta acerca de la poliomielitis o la vacunación antipolio. por favor hágala ahora mismo. a llame a su médico o su Departamento de Salud antes de firmar esta forma.

REACCIONES: Si una persona que recibe la vacuna se enferma y visita a un médico. algún hospital o alguna clínica en las primeras 4 semanas después de la vacunación. por favor repórtelo a:

____

FAVOR DE GUARDAR ESTA PARTE DE LA HOJA PARA SU INFORMACION

He leído la información que contiene esta forma acerca de la poliomielitis y la vacuna atenuada. He tenido la oportunidad de hacer preguntas. y éstas fueron contestadas satisfactoriamente. Creo que entiendo los beneficios y los riesgos de la vacuna antipolio atenuada. y solicito que se me administre a mi o a la persona abajo mencionada. a favor de quien tengo la autoridad de hacer esta solicitud.

IP 10/1/80

INFORMACION SOBRE LA PERSONA A QUE RECIBIRA LA VACUNA
(Por favor use letra de imprenta en las primeras tres lineas)

Nombre (apellido) (primer) (segundo) Fecha de nacimiento Edad

Dirección Condado de residencia

Ciudad Estado Zip Code

X

Firma de la persona que recibirá la vacuna o de la persona autorizada para solicitarla. Fecha

PARA EL USO DE LA CLINICA

Identidad de la clínica

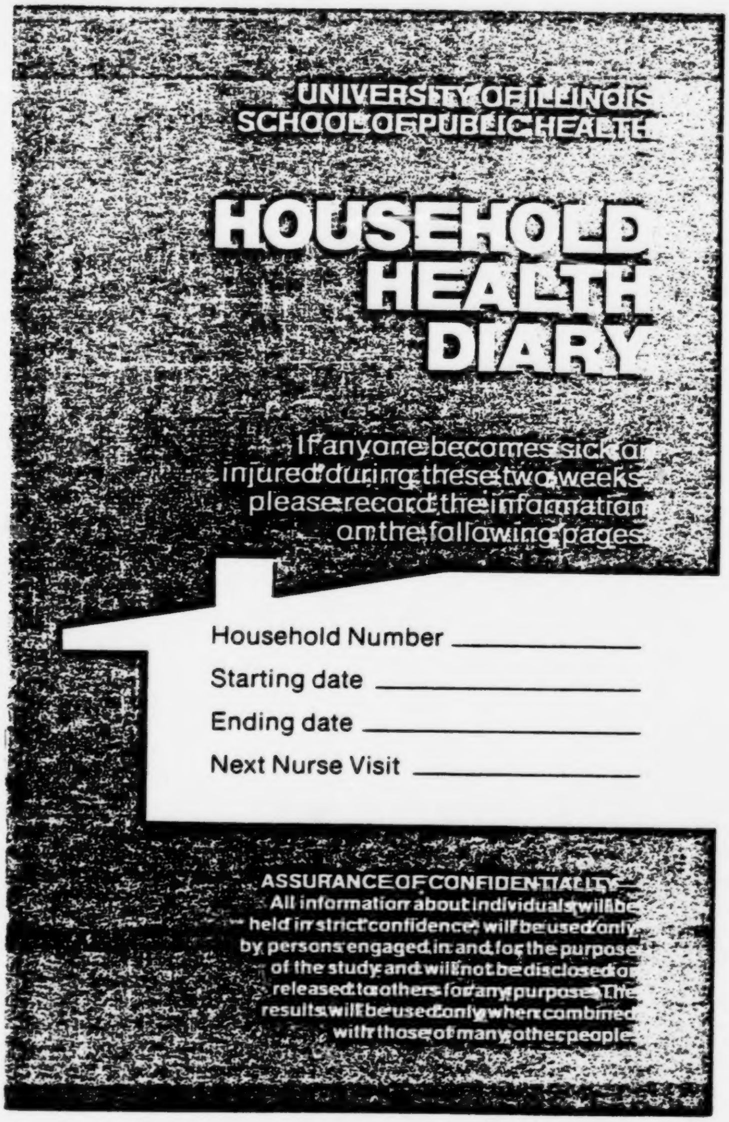
Fecha de vacunación

Fabricante y nº de lote

Lugar de la inyección

APPENDIX F

HOUSEHOLD HEALTH DIARY BOOKLET (1980)



UNIVERSITY OF ILLINOIS
SCHOOL OF PUBLIC HEALTH

HOUSEHOLD HEALTH DIARY

If anyone becomes sick or injured during these two weeks please record the information on the following pages.

Household Number ______________

Starting date ______________

Ending date ______________

Next Nurse Visit ______________

ASSURANCE OF CONFIDENTIALITY—All information about individuals will be held in strict confidence, will be used only by persons engaged in and for the purpose of the study and will not be disclosed or released to others for any purposes. The results will be used only when combined with those of many other people.

Reproduced from best available copy.

GENERAL INSTRUCTIONS FOR KEEPING THIS DIARY

1. Information from this diary can help to determine the health levels of people in your community. Since it is so important, we appreciate your doing your best to make the data as complete as possible. Always make entries in the diary at the time that the event happens so you won't forget.
2. Be sure to include all household members – adults, children and babies. Do not include short-time visitors.
3. Record any notes on page 8.
4. If you have any questions about how to report something in this diary please call:

____

Telephone Number ______________________

or consult the examples which appear below.

DIRECTIONS

1. List all illnesses and injuries during these two weeks for all household members. Even the slightest cold, cough or cramps should be reported.
2. If the same person gets sick, stays home for two or three days, feels better and returns to work, then stays home again, you would record this illness twice.
3. If anyone in your household visits a doctor, note that in the appropriate box in the diary. Then, on the back cover of the diary, please indicate that doctor's name and the town in which he is located.
4. If a household member plans to be out of the study area for longer than 5 days, note this on the back cover of the diary.

SAMPLE

Date illness began	Date of recovery	Who in the family? (first name)	What was his/her illness?	How many days did he/she		Did they . . . (check all that apply)			
				Feel ill but do usual tasks?	Miss work or school?	Call or visit a doctor?	Take any over the counter drugs?	Take any prescription medicine?	Become hospital-ized?
FEB 2	FEB 5	Joe	Cold	3	1	✓	✓		
FEB 3	FEB 7	Susan	Flu	4	2		✓		
FEB 10	FEB 11	David	Sprained Ankle	2	0				
FEB 12	FEB 14	Joe	Cold + Cough	3	3	✓	✓	✓	

Date illness began	Date of recovery	Who in the family? (first name)	What was his/her illness?

How many days did he/she		Did they . . . (check all that apply)			
Feel ill but do usual tasks?	Miss work or school?	Call or visit a doctor?	Take any over the counter drugs?	Take any prescription medicine?	Become hospitalized?

APPENDIX G

HEALTH DIARY FORMS AND WEEKLY ILLNESS SURVEILLANCE SUMMARY (1982 AND 1983)

A. *First week of data collection period*

1. Since I last called you, has anyone in the household had a cold, sore throat, flu or any other respiratory illness?

   1. Yes *(Enter information below)* 2. No

2. Since I last called you, has anyone in the household had any stomach or abdominal illness?

   1. Yes *(Enter information below)* 2. No

3. Since I last called you, has anyone in the household had any skin conditions?

   1. Yes *(Enter information below)* 2. No

4. Since I last called you, has anyone in the household had any eye or ear conditions?

   1. Yes *(Enter information below)* 2. No

5. Since I last called you, has anyone in the household had any other kinds of illnesses or conditions?

   1. Yes *(Enter information below)* 2. No

6. Since I last called you, has anyone in the household been away from the area for more than two days, or returned home after an extended absence?

   1. Yes *(Enter information below)* 2. No

I D	Date illness began	Date of recovery	Who in the family? (first name)	What was his/her illness?	Code	How many days did he/she		Did they . . . (check all that apply)			
						Feel ill but do usual tasks?	Miss work or school?	Call or visit a doctor?	Take any over the counter drugs?	Take any prescription medicine?	Become hospitalized?

1st Contact Attempt________________

2nd Contact Attempt________________

3rd Contact Attempt________________

Respondent______________________

Interviewer______________________

B. *Second week of data collection period*

1. Since I last called you, has anyone in the household had a cold, sore throat, flu or any other respiratory illness?

   1. Yes *(Enter information below)* 2. No

2. Since I last called you, has anyone in the household had any stomach or abdominal illness?

   1. Yes *(Enter information below)* 2. No

3. Since I last called you, has anyone in the household had any skin conditions?

   1. Yes *(Enter information below)* 2. No

4. Since I last called you, has anyone in the household had any eye or ear conditions?

   1. Yes *(Enter information below)* 2. No

5. Since I last called you, has anyone in the household had any other kinds of illnesses or conditions?

   1. Yes *(Enter information below)* 2. No

6. Since I last called you, has anyone in the household been away from the area for more than two days, or returned home after an extended absence?

   1. Yes *(Enter information below)* 2. No

I D	Date illness began	Date of recovery	Who in the family? (first name)	What was his/her illness?	Code	How many days did he/she		Did they . . . (check all that apply)			
						Feel ill but do usual tasks?	Miss work or school?	Call or visit a doctor?	Take any over the counter drugs?	Take any prescription medicine?	Become hospital-ized?

1st Contact Attempt__________________

2nd Contact Attempt__________________

3rd Contact Attempt__________________

Respondent________________________

Interviewer_______________________

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A. *First week of data collection period*

AFFIX LABEL HERE

1st contact attempt ________
2nd contact attempt ________
3rd contact attempt ________
Respondent ________
Interviewer ________

I am calling to get health watch information from you (your household) for last week, beginning Sunday (date) and ending Saturday (date). During that time have you (or any member of your family) had any of the following illnesses?

1. Cold, sorethroat, flu or any other respiratory illness?

   YES (Enter information below) *NO*

2. Any stomach or abdominal illnesses?

   YES (Enter information below) *NO*

3. Any skin conditions?

   YES (Enter information below) *NO*

4. Any eye or ear conditions?

   YES (Enter information below) *NO*

5. Any other kinds of illnesses or conditions?

   YES (Enter information below) *NO*

6. Since I last talked with you, has anyone in the household been away from the area for more than two days or returned home after an extended absence?

   YES (Enter information below) *NO*

7. *(Ask only of households not located on the Hancock Farm)*

   Have you (or any member of your household) spent more than 30 minutes on the Hancock Farm this week?

   YES (Enter information on Wastewater Exposure Sheet) *NO*

3. *(Ask all Hancock Farm residents and non-residents who answered YES to Question #7.*

   A. Did anyone in the household have direct contact with the wastewater?

   YES (Enter information on Wastewater Exposure Sheet) *NO*

   B. Was anyone exposed to the mist or the aerosol from an operating spray rig?

Date illness began	Date of recovery	Who in the family? (first name)	What was his/her illness?	How many days did he/she		Did they . . . (check all that apply)			
				Feel ill but do usual tasks?	Miss work or school?	Call or visit a doctor?	Take any over the counter drugs?	Take any prescription medicine?	Become hospitalized?

B. *Second week of data collection period*

AFFIX LABEL HERE

1st contact attempt ________

2nd contact attempt ________

3rd contact attempt ________

Respondent ________

Interviewer ________

1. Since I last called you, has anyone in the household had a cold, sore throat, flu or any other respiratory illness?

   Yes *(Enter information below)* No

2. Since I last called you, has anyone in the household had any stomach or abdominal illness?

   Yes *(Enter information below)* No

3. Since I last called you, has anyone in the household had any skin conditions?

   Yes *(Enter information below)* No

4. Since I last called you, has anyone in the household had any eye or ear conditions?

   Yes *(Enter information below)* No

5. Since I last called you, has anyone in the household had any other kinds of illnesses or conditions?

   Yes *(Enter information below)* No

6. Since I last called you, has anyone in the household been away from the area for more than two days, or returned home after an extended absence?

   Yes *(Enter information below)* No

7. Since I last called you, has anyone in the household had any contact whatsoever with wastewater on the Hancock farm (i.e., wastewater on shoes; clothes; skin or hair; eyes or mouth) ? *If any wastewater contact is reported, record type of contact and brief explanation of how contact occurred.*

   Yes *(Enter information below)* No

Date illness began	Date of recovery	Who in the family? (first name)	What was his/her illness?	How many days did he/she		Did they . . . (check all that apply)			
				Feel ill but do usual tasks?	Miss work or school?	Call or visit a doctor?	Take any over the counter drugs?	Take any prescription medicine?	Become hospitalized?

NUMBER OF NEW ACUTE ILLNESSES REPORTED IN STUDY POPULATION BY WEEK - 1982 *
(BASED ON PHONE INFORMATION FROM FIELD REPRESENTATIVES)

DCP	STARTING DATE	# OF PARTICIPANTS REPORTING	RESPIRATORY	GI	EYE & EAR	SKIN	OTHER ACUTE	OTHER CONDITIONS
201	1-3	392 (54)	26 (0)	--	--	--	--	3 (0)
201	1-10	380 (54)	17 (1)	1 (0)	--	--	--	2 (0)
202	1-17	389 (54)	11 (0)	1 (0)	--	4 (0)	--	--
202	1-24	387 (54)	16 (3)	5 (0)	1 (0)	--	2 (0)	4 (0)
203	1-31	382 (54)	18 (1)	7 (2)	--	--	--	--
203	2-7	379 (54)	6 (2)	2 (0)	--	2 (0)	--	2 (0)
204	2-14	379 (53)	15 (3)	5 (0)	1 (0)	2 (0)	1 (1)	2 (0)
204	2-21	379 (53)	12 (3)	8 (0)	1 (0)	--	1 (0)	3 (0)
205	2-28	377 (53)	3 (1)	1 (0)	--	1 (0)	1 (0)	1 (0)
205	3-7	374 (53)	18 (0)	--	--	--	2 (0)	1 (0)
206	3-14	367 (48)	10 (0)	2 (0)	2 (0)	--	2 (0)	--
206	3-21	351 (49)	9 (0)	6 (0)	--	3 (0)	--	--
207	3-28	374 (50)	12 (0)	--	--	--	--	1 (0)
207	4-4	367 (50)	15 (6)	4 (0)	--	--	--	2 (0)

* NUMBERS IN PARENTHESIS ( ) INDICATE ILLNESSES OCCURRING IN ZONE I

APPENDIX H

ACTIVITY DIARIES AND MAPS

School of Public Health
UNIVERSITY OF ILLINOIS AT THE MEDICAL CENTER, CHICAGO

Area Code 312, Telephone 996-6620
Mailing Address: P.O. Box 6998 · Chicago, Illinois 60680

March 11, 1982

Dear Study Participant:

In order for us to get a better understanding of the relationship between all of the environmental and health data which are being collected, it is necessary for us to know how much time individuals spend in various parts of the study area. Obviously, it would be impossible for you to keep track of your whereabouts every day that we are collecting health information, so we have developed an "activity diary" which we would like study participants to keep for one week. We hope that this week will be representative of people's normal activities at this time of year.

We are asking that each member of your household complete an activity diary for the week of March 21 - 27. Each person should fill out the activity diary with his or her name on it each day for the one week period. (Mothers should fill out the diary for young children.)

Included with the diary is a map of the study area with different colored sections on it. This map should be used when answering question 1. If you live or spend time within the city of Wilson, you may also need to use the enlarged map of Wilson in order to distinguish exactly where the boundaries between the orange and white areas are. When answering question 1, try to record as accurately as possible the number of hours spent in the various areas each day. For example, if you live in the orange area and spend only 10 minutes driving through the blue area on a particular day, it would not be necessary to record the 10 minutes spent in the blue area. If, however, you spend half an hour or more in any of the areas, that time should be recorded.

Question 2 requests more specific information as to how much time is spent in Lubbock or at home. "At home", in this case, means that you are either in your house, yard, or barnyard area. For both questions, if you do not spend any time in a certain area, please mark a "0" in the column, instead of leaving it blank.

If there are college students or other family members in your household who normally spend most of their time away from the area, an activity diary should still be completed for them during the week of March 21st. The time during which they are away from home would simply be recorded as "hours outside map area". If there is someone in your household who is usually at home, but just happens to be gone all or most of the week of the 21st, that person should complete the activity diary the first week that he or she returns home.

The activity diaries should be returned to the University of Illinois in the enclosed stamped, self-addressed envelope as soon as they are completed.

We hope that filling out the activity diary will not be too much of an inconvenience. The information which the diary will provide is crucial to the health study, and we greatly appreciate your efforts in completing it.

Sincerely yours,

Robert Northrop, Ph.D.
Associate Professor
Epidemiology-Biometry Program

RN/cb

ACTIVITY DIARY

A. Basic Data

1. Name: ______________ ______________
First Last

2. Reporting week dates: ______ to ______

B. Activity Information

Please record the number of hours per day which you spend within each area (column) listed below. Use the reference maps to locate the areas for question 1; question 2 refers to specified locations familiar to you. This should be done each day for one week. If you are out-of-town during the entire week, please complete this diary the first week that you return home.

Don't forget to include sleeping hours when you record daily activities. The number of hours for each day should total 24 hours.

Question 1: How many hours per day did you spend in the following areas?

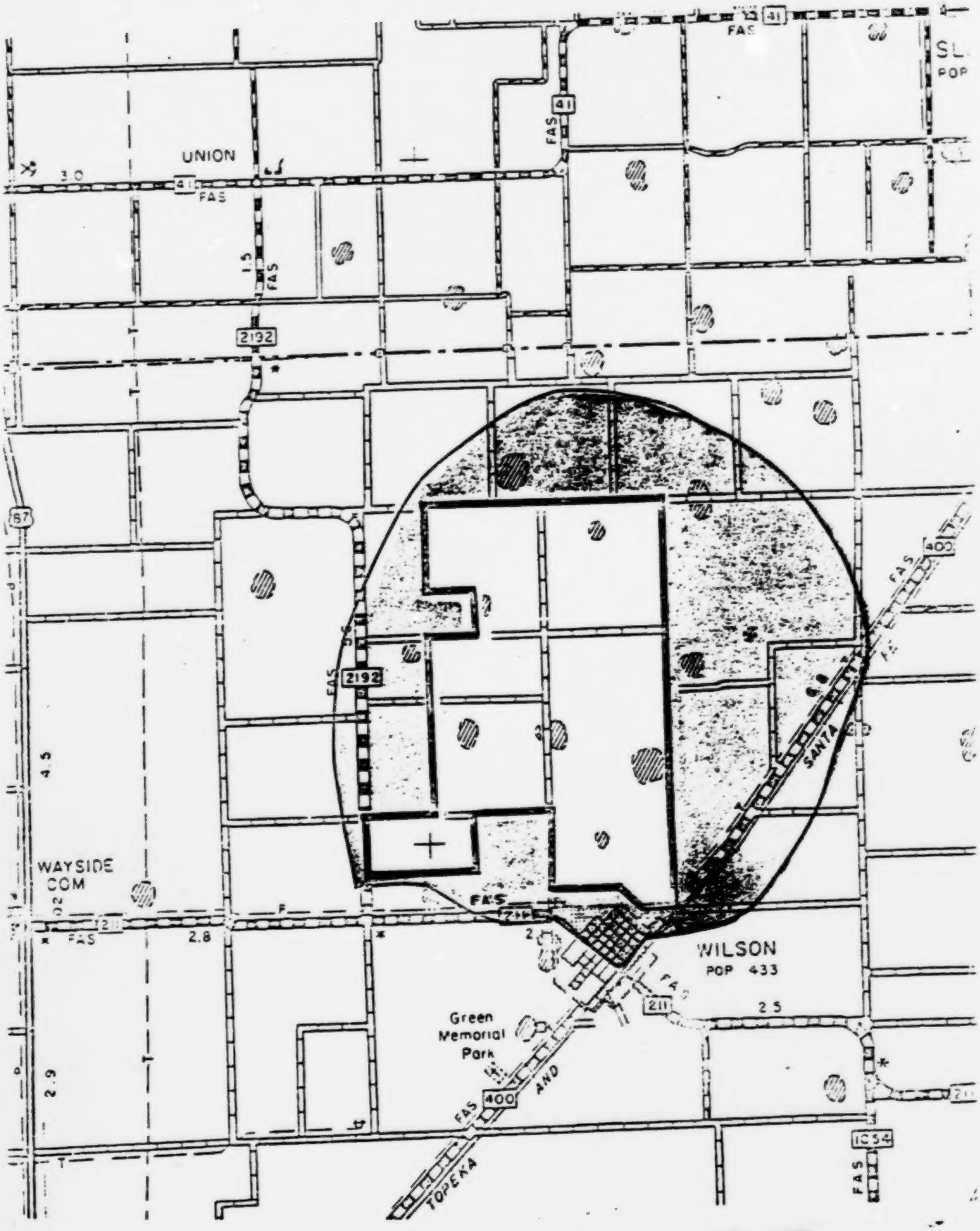
HOURS PER DAY

	Blue Map Area (Hancock farm)	Orange Map Area	White Map Area	Outside Map Area	Daily Total (24 hrs.)
Sunday	______	______	______	______	______
Monday	______	______	______	______	______
Tuesday	______	______	______	______	______
Wednesday	______	______	______	______	______
Thursday	______	______	______	______	______
Friday	______	______	______	______	______
Saturday	______	______	______	______	______

Question 2: In addition to the above, we would also like more detailed information as to how many hours per day you spent in the following specific locations.

HOURS PER DAY

	In Lubbock	At Home
Sunday	______	______
Monday	______	______
Tuesday	______	______
Wednesday	______	______
Thursday	______	______
Friday	______	______
Saturday	______	______



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POP
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FAS
UNION
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FAS
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2.8
FAS
211
WILSON
POP 433
FAS
211
2.5
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Memorial
Park
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AND
TOPEKA
SANTA
FE
FAS
400
1054
FAS
2.9

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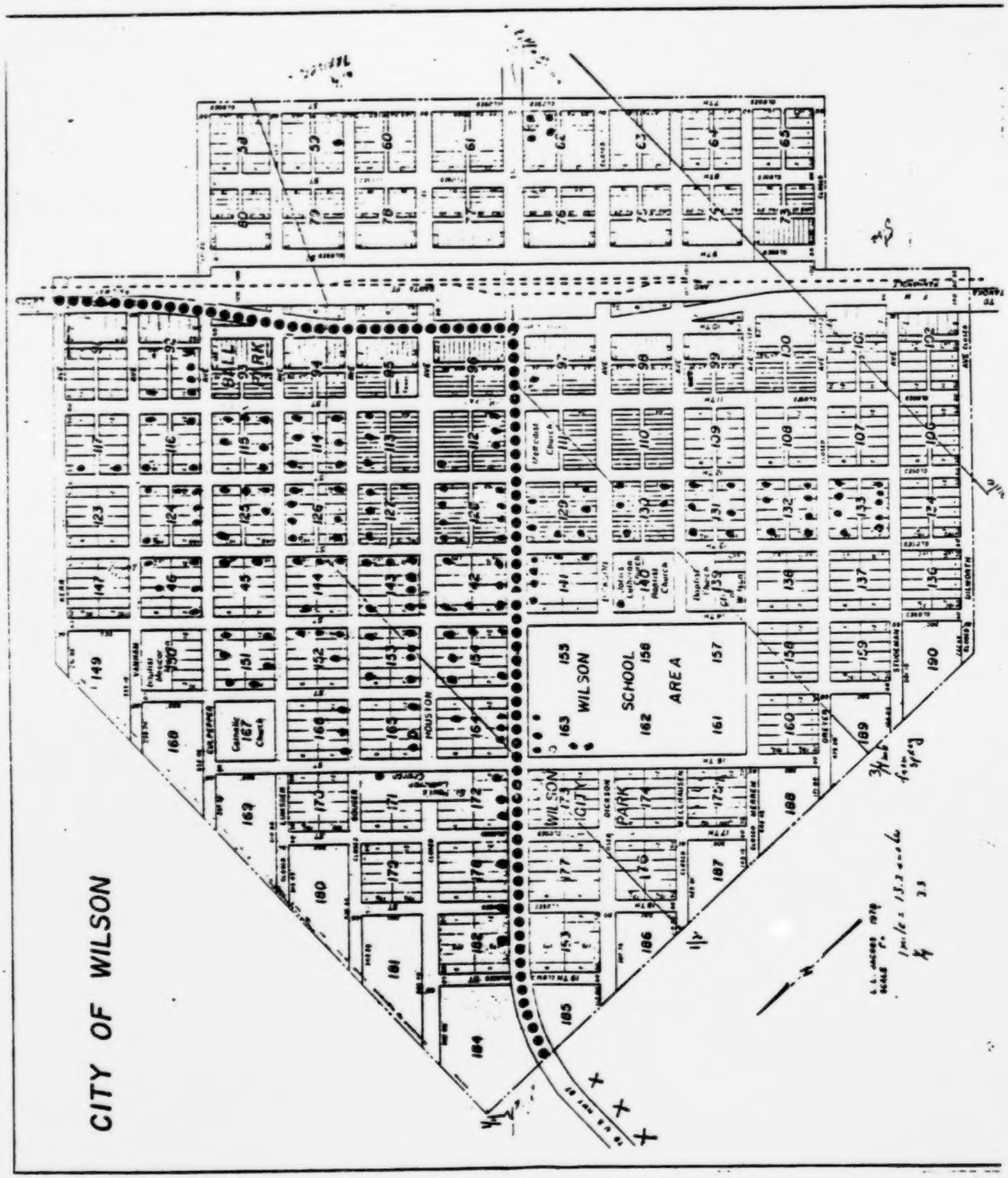

CITY OF WILSON
BALL PARK
WILSON SCHOOL AREA
WILSON CITY PARK
Catholic Church 167
Baptist Church
Lutheran Church
HOUSTON
DILWORTH
STUDEMAN
CULPEPPER
DICKSON
WILLIAMSEN
GREVER
LANGHAM

School of Public Health
UNIVERSITY OF ILLINOIS AT THE MEDICAL CENTER, CHICAGO

Area Code 312, Telephone 996-6620
Mailing Address: P.O. Box 6998 · Chicago, Illinois 60680

July 27, 1982

Dear Study Participant:

I believe you are familiar with the procedure for keeping the activity diary, so I will not restate all the directions we have given to you previously. There are 3 important points about this diary:

1. Please keep the diary for the week of August 1st through 7th;
2. The completed activity diaries will be collected when fecal specimens are collected during the week of August 9 through August 13. The diaries can either be brought to the Wilson Mercantile Building or arrangements can be made to pick up these diaries at your home by calling Pearl Davidson (628-2961);
3. Please be sure to use the enclosed maps when you refer to times spent in the colored areas. These maps are different from previous activity diary maps.

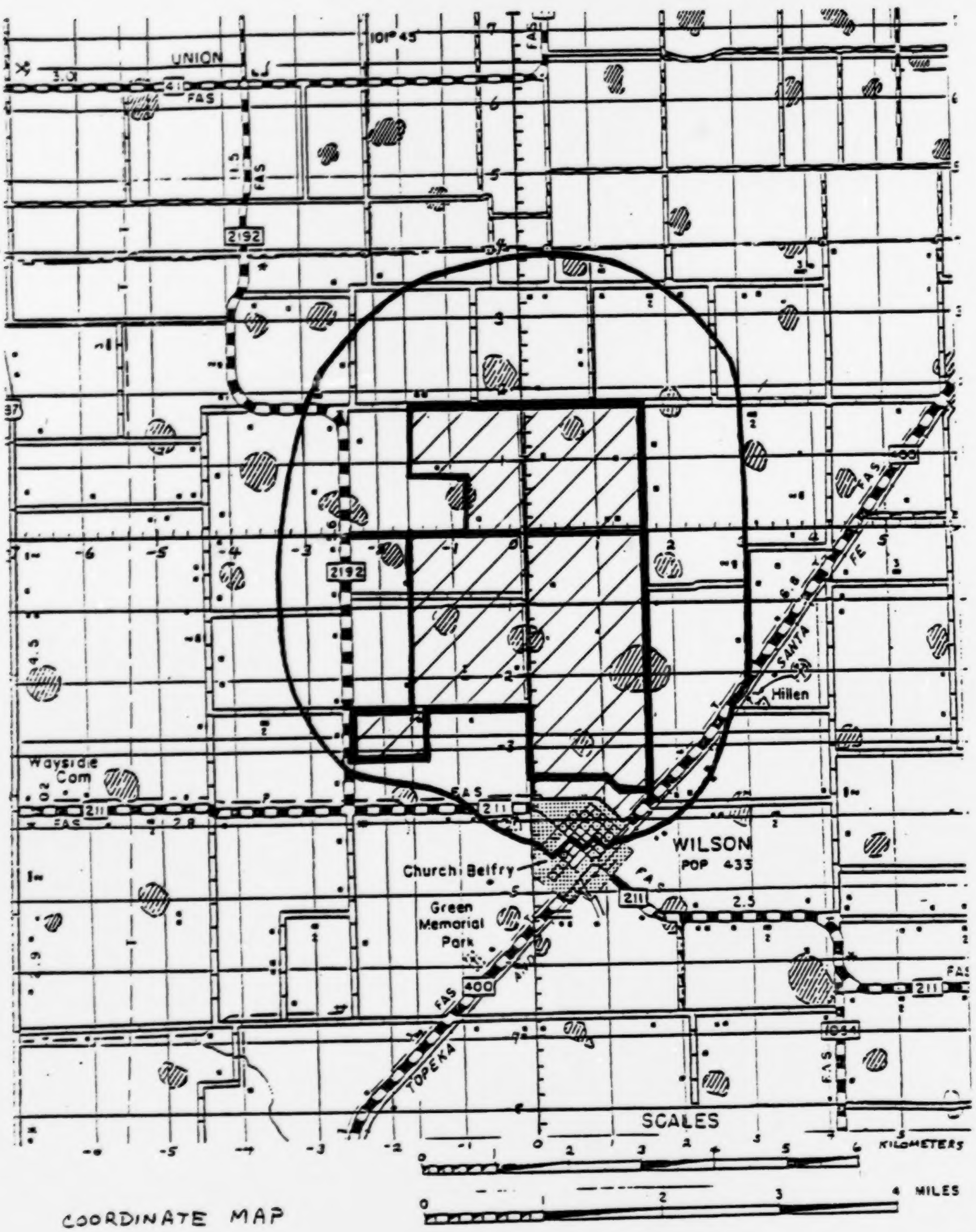
If you have difficulty in keeping this diary, Parrie Graham will be glad to answer your questions when she is at the Wilson Mercantile Building (628-2621) during the week of August 9 - 13.

This diary is particularly important to us since your activities may be very different from previous times, particularly those of you who would have been doing more farming than has been possible this year.

We really do appreciate your time in doing this task for us.

Sincerely yours,

Robert Northrop, Ph.D.
Associate Professor
Epidemiology-Biometry Program


101°45
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Green Memorial Park
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COORDINATE MAP

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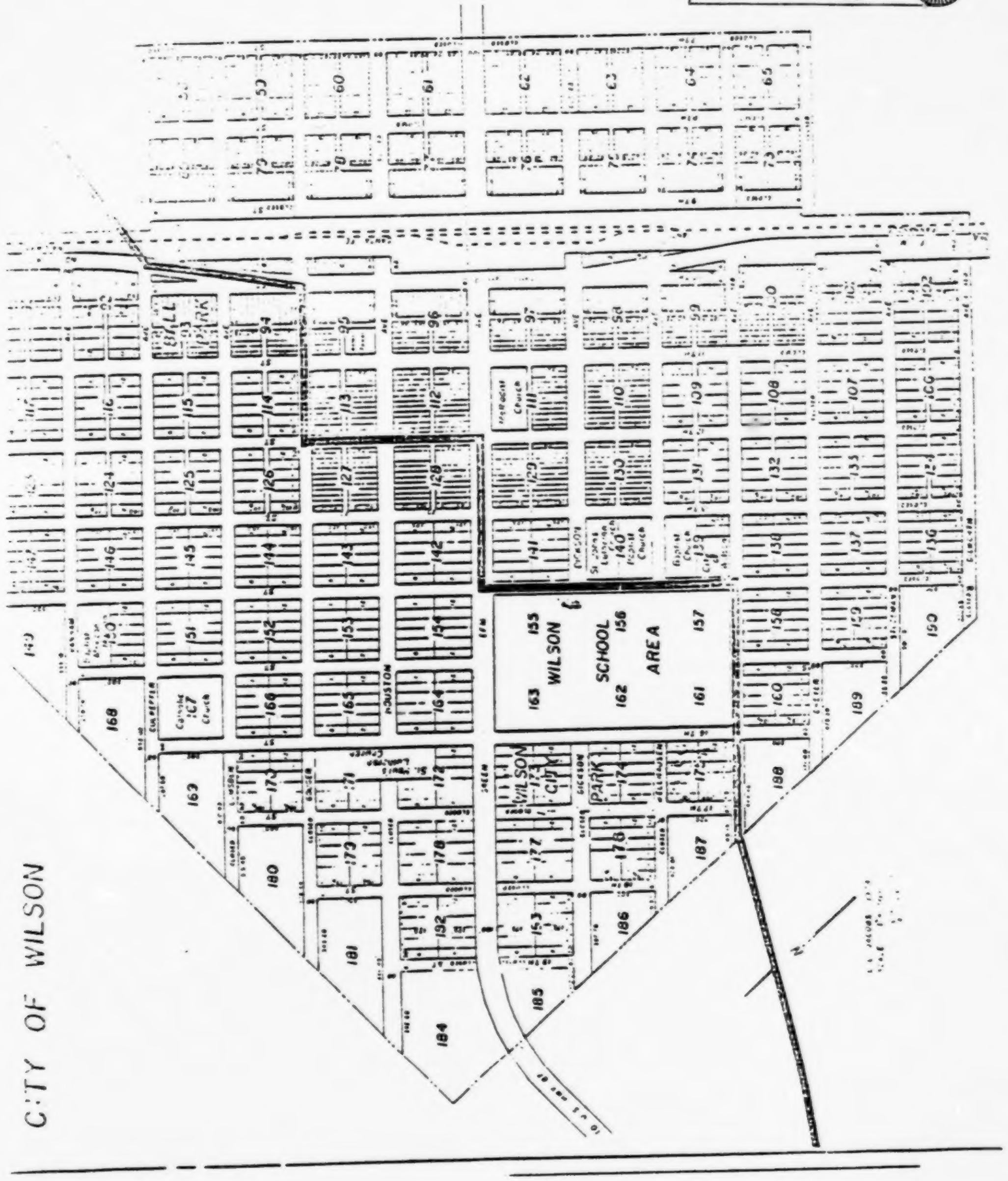
CITY OF WILSON
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WILSON CITY PARK
HOUSTON
Catholic 167 Church
Baptist Church
Methodist Church
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APPENDIX I

WILSON EATING ESTABLISHMENT SURVEY FORM

NAME________________________________

ID________________________________

Did you/_______ eat any food which was prepared at any of the establishments in Wilson during 1982 or 1983 ?

0 NO
1 YES

Which Establishment?		Year	Frequency in the summer compared to rest of year	Summer Frequency
Restaurant A	1982	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	
	1983	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	
Restaurant B	1982	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	
	1983	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	
Restaurant C	1982	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	
	1983	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	
Restaurant D	1982	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	
	1983	0 no	1 more	1 1+ times/week
		1 yes	2 same	2 1/week to 1/month
			3 less	3 1 to 3 times/summer
			4 never	

APPENDIX J

PROCEDURE FOR WASTEWATER SAMPLE COLLECTION, LUBBOCK SOUTHEAST WATER RECLAMATION PLANT

PROCEDURE FOR WASTEWATER SAMPLE COLLECTION

Operational Year - 1981
Trickling Filter Effluent - Lubbock Southeast Water Reclamation Plant
SwRI Project 01-6001

Purpose -

The purpose for collection of this sample is to determine relative densities of a wide range of indigenous enteric bacteria and viruses prevalent in the wastewater to be land applied at the Hancock site. To accomplish this purpose a 24-hour flow-weighted composite is derived by collecting three eight-hour time-weighted samples from the Trickling Filter Plant (TFP) effluent followed by compositing based on plant flow data for each eight-hour period.

Equipment Required -

Sample Collection -

- ISCO Model 1580 Sampler with Nicad battery
- 109 ft. (3 m) of 3/8" O.D. x 1/4" I.D. Tygon tubing
- Weighted stainer
- 3 clean 3-gallon polyethylene containers (for ISCO)
- 10 to 20 lbs. cracked or cube ice (function of ambient conditions)

Sample Compositing -

- 5-gallon Nalgene (or requivalent) polypropylene carboy with lid (sterile)
- 1-liter Nalgene (or equivalent) graduated cylinder (sterile)
- 1-liter Nalgene polypropylene bottles (sterile)

Sample Shipment

- 1 frozen Kool-Pac per six 1-liter sample bottles
- 1 insulated shipping container, labeled, with means of lid attachment
- 1 counter-to-counter shipping ticket (Southwest or Braniff Airlines)

Procedure

Preparation

1. Charge two Nicad batteries for 24 hours prior to sample collection.
2. Check equipment for completeness including new Tygon tubing with weighted strainer securely attached.
3. Place Kool-Pacs in freezer at least 24 hours prior to sample compositing.
4. Sterilize equipment for compositing as appropriate.

Sample Collection -

1. Locate sample adjacent to combined channel from the secondary clarifiers of the TFP.

2. Place a 3-gallon container in the Sample Container Tub with the false bottom open end up. Carefully add crushed or cube ice to the tub without disturbing the position of the container.

3. Replace the Pump and Controls Section and latch securely making sure that the Stop Float Mechanism is free. Attach the battery to the sampler and securely connect the battery cable to the "12 VDC" socket on the side of the control box. Attach the Tygon tubing to the pump inlet, tape to secure, and lower weighted strainer into the effluent channel. Tape tubing to side of sampler to reduce strain on pump inlet connection.

4. Set the Control Panel as follows:

   Mode Switch - Time
   Time Interval Multiplier Control - 1.0
   Suction Line Length Switch - 14 2/3' (1/4" I.D.)
   Sample Rate Switch - 10 min.
   Volume Selector Switch - 268 mL/sample (8' head)
   Pump Switch - Auto

5. Turn Sample Rate Switch to the Manual Cycle position, then return it to the 10 min. Time Inverval Position. The pump should be automatically activated, first for a brief period in the reverse mode to purge any liquid in the line followed by a forward pumping action of sufficient time to collect approximately 268 mL of sample. This cycle is completed by a second reverse pumping opertaion to again purge the sample line. If all functions operate correctly in this test cycle, confirm the position of all control switches, especially that the Pump Switch is in the Auto Mode, then place and latch both the protective lid over the Control Panel and the cover over the Pump and Controls Section. Refer to the instruction manual should problems be encountered.

6. Check the TFP Flow meter in the treatment plant office for operation, and if necessary, mark the chart for start of sample collection.

7. At the end of each 8-hour sampling period, turn the Pump Switch to Off, remove the 3-gallon sample container, label it, and place a clean sample container in the tub. Turn the Pump Switch to the Auto position and repeat Step 5. Renew the ice bath as required to maintain the collected sample at 4°C. Store the collected sample at 4°C until composited. At the conclusion of the 24-hour sampling period, remove all equipment from the sampling site.

8. Prior to leaving the treatment plant obtain information on TFP flows as follows:
    (a) Remove the TFP flow chart recorder from the instrument panel after disconnecting the multi-lead sockets at the back.
    (b) On a clean work table carefully unroll sufficient chart paper from the take-up chart spool to correspond to the 24-hour collection period. Mark the 8-hour intervals, and using a transparent straight edge, pencil a horizontal line through a visually estimated average for each 8-hour segment. Record average flows for each segment.
    (c) Rewind the chart paper on the take-up spool to the correct time, replace the recorder in the instrument panel, and connect the multi-lead sockets. Confirm that the recorder is functional.

Sample Compositing

1. Based on total flow through the TFP during the 24-hour composite period (Σ of the average flow for each 8-hour sample segment), determine the fraction of total flow for each segment.
2. Knowing the final volume of composite desired (18 L max for 5-gal. jug), determine the amount of sample needed for each segment based on the fraction of total flow for that segment (final volume desired X fraction of total flow).
3. Add appropriate amounts of each sample to the sterile composite container using a sterile 1-L graduate. Cap and shake to mix.
4. Apply sample labels to sterile, 1-L polypropylene bottles and cover with a complete circle of clear protection tape.
5. Transfer composite sample to 1-L bottles and cap tightly.

Sample Shipment

1. Samples should be shipped at 4°C. If samples are not at this temperature and the shipping schedule permits, place samples in a 4°C environment (refrigerator or ice bath) prior to packing.
2. Pack a shipping container with the sample bottles. Add a frozen Kool-Pac to the container insulating the sample containers where necessary to prevent direct contact between container and Kool Pac.

3. Close container and strap securely. Check address label for legibility.

4. Present shipping container with completed shipping ticket at the passenger check-in counter or freight counter of designated airline (Braniff or Southwest) at lease 45 minutes prior to scheduled departure. Shipment is to be prepaid.

1/15/81

01-6001

J. Harding

bz:2H

APPENDIX K

PROCEDURE FOR WASTEWATER SAMPLE COLLECTION, WILSON IMHOFF TANK EFFLUENT

PROCEDURE FOR WASTEWATER SAMPLE COLLECTION

Operation Year - 1981
Wilson, Texas Imhoff Tank Effluent
SwRI Project 01-6001

Purpose -

The purpose for collection of this sample is to determine relative densities of a wide range of indigenous enteric bacteria and viruses prevalent in the wastewater from the Wilson community, the most densely populated area adjacent to the Hancock Site. To accomplish this purpose a 24-hour time-weighted composite sample is collected by utilizing a self-contained automatic sampler.

Equipment Required -

Sample Collection -
- ISCO Model 1580 Sampler with Nicad battery
- 6 ft. (2m) of 3/8" O.D. x 1/4" I.D. Tygon tubing
- Short length of pipe for tubing weight
- 1 clean 3-gallon polyethylene container for ISCO
- 10 to 20 lbs cracked or cube ice (function of ambient conditions)

Sample Shipment -
- 1 frozen Kool-Pac per 6 (six) 1-liter sample bottles
- 1 insulated shipping container, labeled, with means of lid attachment
- 1 counter-to-counter shipping ticket (Southwest or Braniff Airlines)

Procedure -

Preparation -
1. Charge two Nicad batterys for 24 hours prior to sample collection. If this sample is collected simultaneously with the Trickling Filter Effluent from the Lubbock Southeast Reclamation Plant, only one extra Nicad battery needs to be charged.

2. Check equipment for completeness including new Tygon tubing with weight attached to end.

3. Place Kool-Pacs in freezer at least 24 hours prior to sample shipment.

Sample Collection -
1. Locate sampler adjacent to Imhoff tank effluent drain.

2. Place a 3-gallon container in the Sample Container Tub with the false bottom open and up. Carefully add crushed or cube ice to the tube without disturbing the position of the container.

3. Replace the Pump and Controls Section and latch securely making sure that the Stop Float Mechanism is free. Attach the battery to the sampler and securely connect the battery cable to the

"12 VDC" socket on the socket on the side of the control box. Attach the Tygon tubing to the pump inlet, tape to secure, and lower weighted end into the Imhoff tank drin. Tape tubing to side of sampler to reduce strain on pump inlet connection.

4. Set the Control Panel as follows:

   Mode Switch - Time
   Time Internal Multiplier Control - 1.0
   Suction Line Length Switch - 7 1/3' (1/4" I.D.)
   Sample Rate Switch - 136 mL/sample (8' head)
   Pump Switch - Auto

5. Turn Sample Rate Switch to the Manual Cycle position, then return it to the 10 min. Time Internal position. The pump should be automatically activated, first for a brief period in the reverse mode to purge any liquid in the line followed by a forward pumping action of sufficient time to collect approximately 136 mL of sample. This cycle is completed by a second reverse pumping operation to again purge the sample line. If all functions operate correctly in this test cycle, confirm the position of all control switches, especially that the Pump Switch is in the Auto Mode, then place and latch both the protective lid over the Control Panel and the cover over the Pump and Controls Section. Refer to the instruction manual should problems be encountered.

6. At the end of the 24-hour sampling period, remove the 3-gallon sample container from the Sample Container Tub, cap and label it, and remove all equipment from the sampling site.

Sample Shipment-

1. After thoroughly mixing the sample, fill the appropriate number of 1-1 bottles and cap tightly.

2. Samples should be shipped at 4°C. If samples are not at this temperature and the shipping schedule permits, place samples in a 4°C environment (refrigerator or ice bath) prior to packing.

3. Pack a shipping container with the sample bottles. Add a frozen Kool-Pac to the container insulating the sample containers where necessary to prevent direct contact between container and Kool-Pac.

4. Close container and strap securely. Check address label for legibility.

5. Present shipping container with completed shipping ticket at the passenger check-in counter or freight counter of designated airline (Braniff or Southwest) at least 45 minutes prior to scheduled departure. Shipment is to be prepaid.

2/02/81
01-6001
J. Harding

APPENDIX L

DESCRIPTION OF LITTON MODEL M HIGH VOLUME AEROSOL SAMPLER

APPENDIX L

DESCRIPTION OF LITTON MODEL M HIGH VOLUME AEROSOL SAMPLER

"The Model M Sampler is designed to continuously collect particulate matter from a large volumetric flow rate of air (approximately 1000 liters/minute) and deposite it into a small amount of liquid (flow rate of 2 mL/min). This effects a volumetric concentration factor on the order of 5 x 10^5. Basically, the sampler is an electrostatic precipitator of a rather unusual configuration. With reference to the schematic diagram, Figure L-1, and an interior view, Figure L-2, aerosol is drawn into the unit through a converging nozzle and passes through the center of the high-voltage area. It then flows radially between this plate and a lower rotating collection disc. An electric potential of 15,000 volts, which is maintained across a 11/16-inch spacing between the plate and disc, creates two effects: 1) A corona is emitted from a ring of 60 needles that is located concentric to the air inlet. Particles, exposed to air ions created from the corona, acquire an electrical charge. 2) The electric field provides the driving force to precipitate charged particles onto the lower disc.

"Liquid is pumped onto the center of the collection disc and, because of the centrifugal force, forms a thin moving film over the entire disc surface. Particles collected on the film are transported to a rotating collection ring where the liquid is removed by the pickup. Subsequently, the liquid drips into the collection funnel where it is pumped to a receiver located outside the sampler.

"To accommodate a broad range of sampling situations, several variable features are incorporated into the unit. These are:

Air Flow Rate	400 to 1200 liters/minute
Liquid Flow Rate	0 to 8 mL/minute
Disc Speed	0 to 45 rpm
High Voltage	0 to 20 kilovolts

"When the sampler is in operation, the air flow rate is read directly from a calibrated meter on the front panel and is adjusted with a blower control potentiometer (see Figure L-3). Both disc speed ar pump flow rate

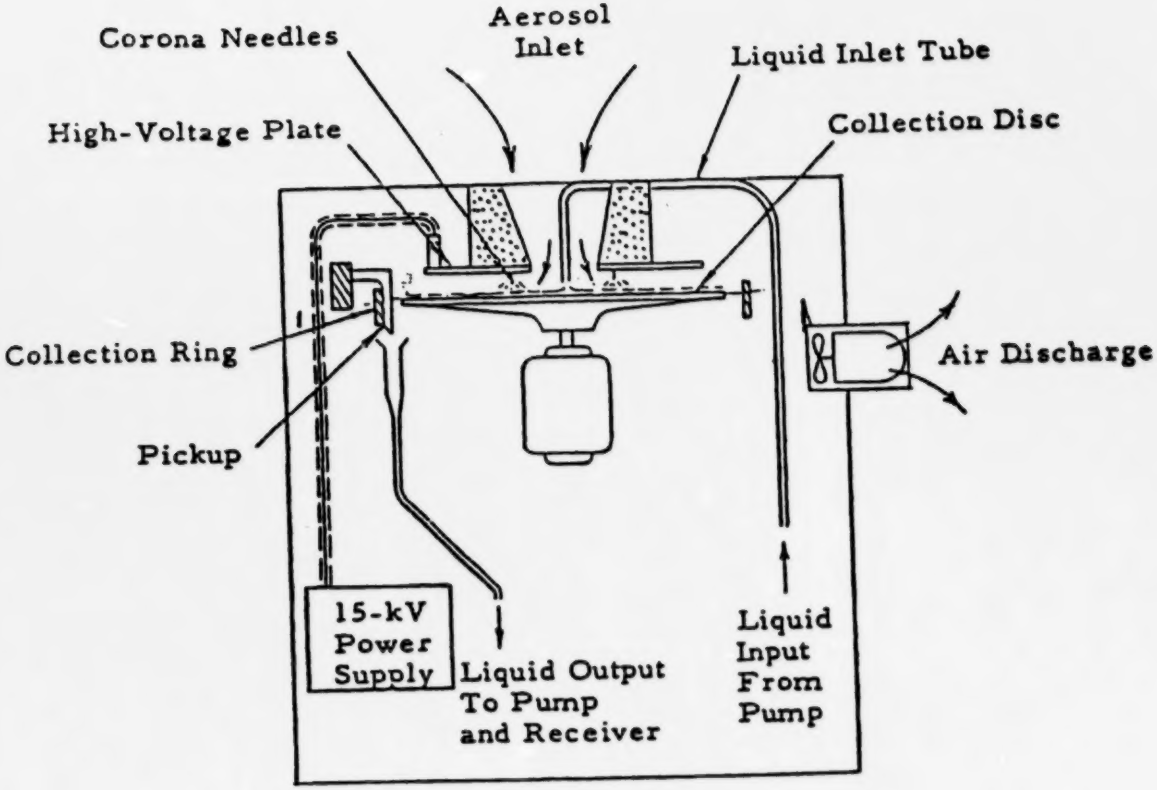

Corona Needles
Aerosol Inlet
Liquid Inlet Tube
High-Voltage Plate
Collection Disc
Collection Ring
Air Discharge
Pickup
15-kV Power Supply
Liquid Output To Pump and Receiver
Liquid Input From Pump

Figure -1.. Schematic Diagram of Large-Volume Air Sampler System

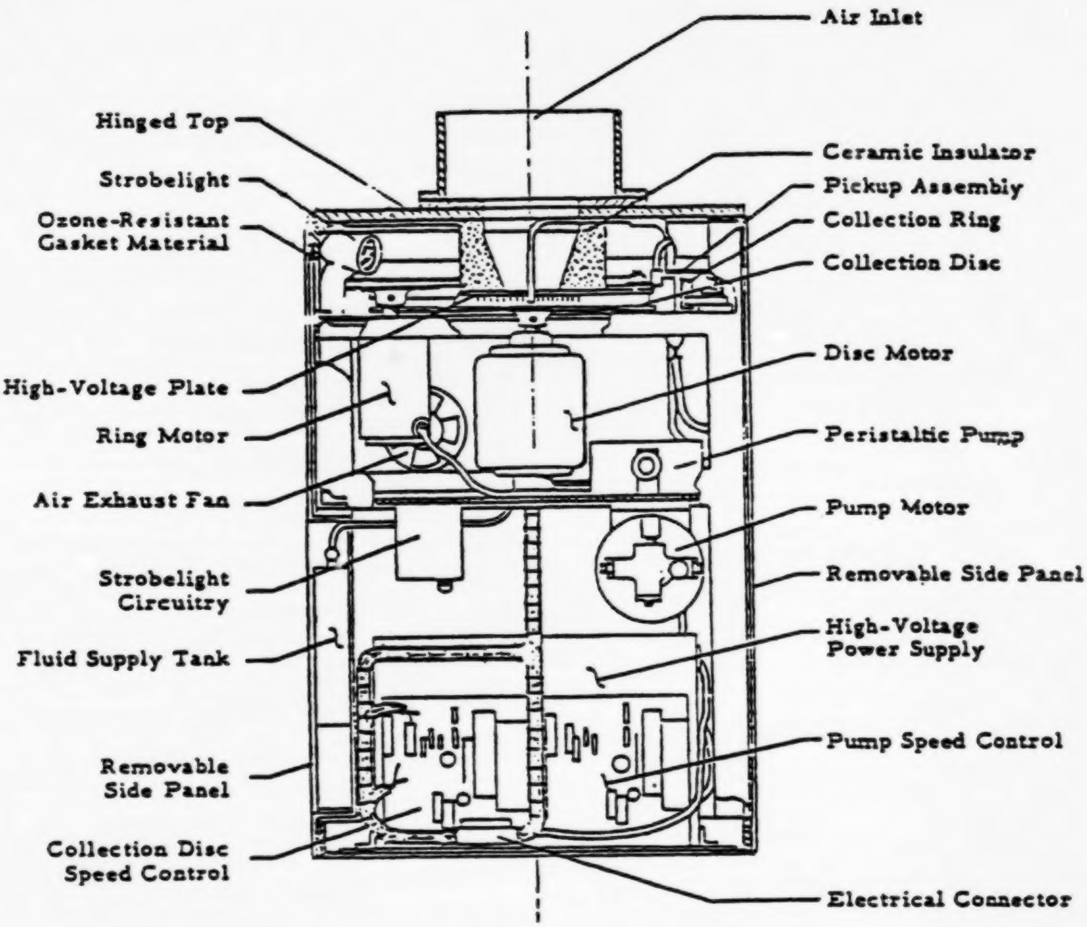
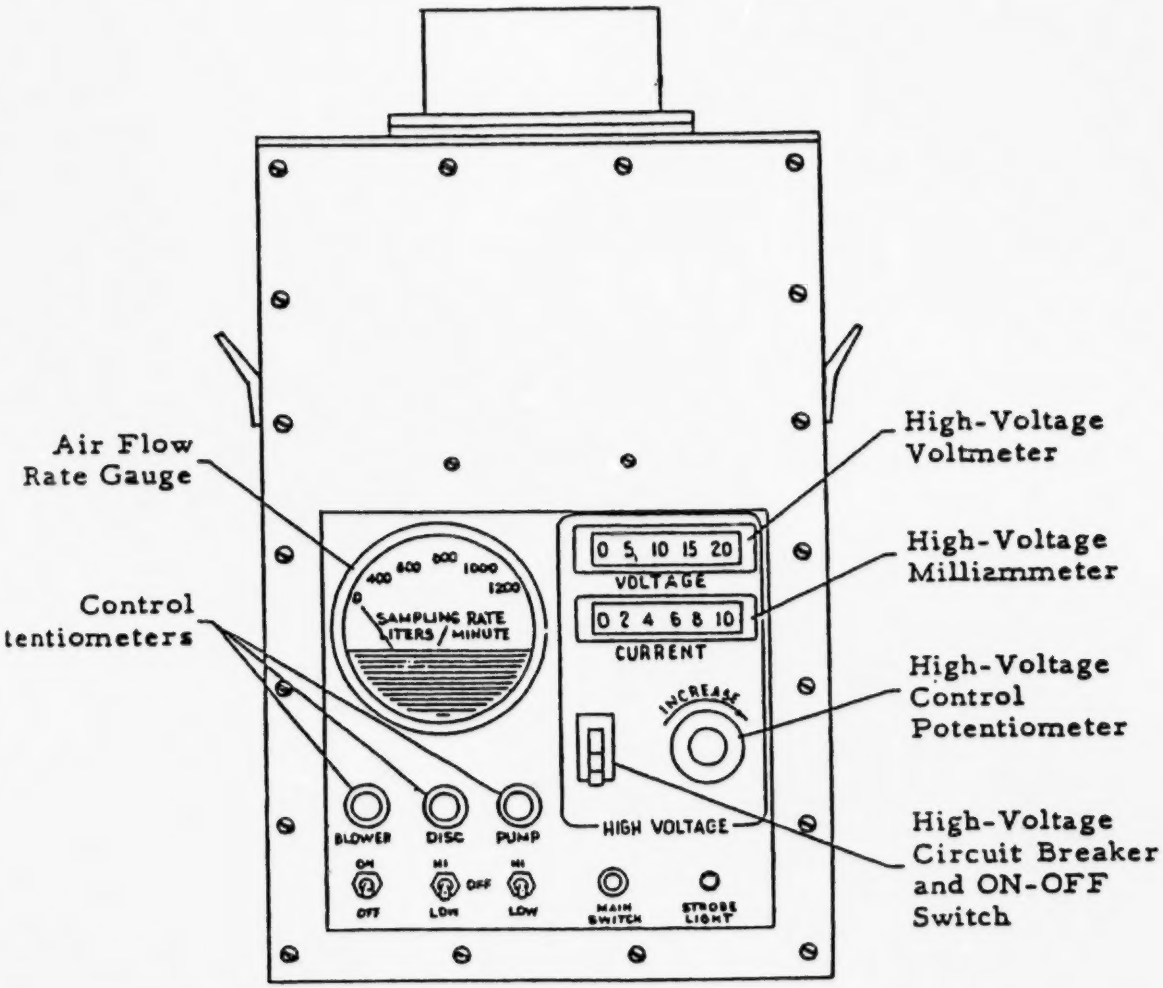

Air Inlet
Hinged Top
Ceramic Insulator
Strobelight
Pickup Assembly
Ozone-Resistant Gasket Material
Collection Ring
Collection Disc
Disc Motor
High-Voltage Plate
Ring Motor
Peristaltic Pump
Air Exhaust Fan
Pump Motor
Strobelight Circuitry
Removable Side Panel
Fluid Supply Tank
High-Voltage Power Supply
Pump Speed Control
Removable Side Panel
Collection Disc Speed Control
Electrical Connector

Figure L-2. Interior View of Large-Volume Air Sampler


Air Flow Rate Gauge
Control tentiometers
High-Voltage Voltmeter
High-Voltage Milliammeter
High-Voltage Control Potentiometer
High-Voltage Circuit Breaker and ON-OFF Switch
0 400 600 800 1000 1200
SAMPLING RATE LITERS / MINUTE
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INCREASE
HIGH VOLTAGE
BLOWER
DISC
PUMP
ON
OFF
HI
OFF
LOW
HI
LOW
MAIN SWITCH
STROBE LIGHT

Figure L-3. Instrument Panel of Model M Large-Volume Air Sampler are controlled by high and low range toggle switches, together with potentiometers. Although no direct readouts are provided for these two variables, calibrations are easily obtained so the arbitrary scales on the potentiometers can be converted to actual speed or flow rates. The high-voltage system is set with the aid of a potentiometer and is provided with the meter to show voltage and current."[1]

To facilitate visual observation of the surface condition of the disc in operation, the operator made observations through the windows with the aid of a flashlight. The air flow rate was set at 1000 liters/minute.

---

1. Litton Model M Large-Volume Air Sampler: Instruction Manual, Report 3028. Minneapolis, Minnesota, 1966.

APPENDIX M

DECONTAMINATION PROCEDURE FOR MODEL M SAMPLERS

APPENDIX M

DECONTAMINATION PROCEDURE FOR LITTON MODEL M

SOLUTIONS:

1% Clorox

$$\text{Buffers--}\left.\begin{array}{ll} KH_2PO_4\ (71\ g/L) & 50\ mL \\ Na_2HPO_4\ (115\ g/L) & 50\ mL \end{array}\right\}\ /L\ DI\ H_2O$$

Autoclave 50 mL of the buffer in 2-oz bottles.
Add 1 mL of 5% Clorox prior to use.

1% sodium thiosulfate
10 g NaThio/L DI H_2O

Sterile water

Autoclave 100 mL in 4-oz bottles prior to use.

PROCEDURE:

1. Calibrate air flow meter for 1000 lpm.
2. Disconnect electrical supply and remove side plate from unit.
3. Using Kimwipes dipped in 70% ethyl alcohol, wipe the inside top half sides and all upper section parts.
4. Run disk (but not blower) and pump 1% Clorox solution through all tubes. Hold Clorox solution in sampler tubing for a minimum of 30 minutes. The pump may be started periodically to move cleaning solution through the tubing.
5. After decontamination with Clorox solution, flush the system with the contents of a sodium thiosulfate bottle.
6. Rinse the system with the contents of a sterile water bottle. After most of the liquid has been pumped out of the system, attach a microfilter to the sampler inlet and run the blower until the disk is dry.
7. Wipe the ends of the tubes with a Kimwipe saturated with 70% ethyl alcohol. Place the ends of the tubes in a clean plastic bag and tape shut. Seal the sampler inlet and exhaust ports with decontaminated plastic caps.

01-6001-313
HJH 2/82

APPENDIX N

COLLECTION EFFICIENCY OF LITTON MODEL M LARGE VOLUME SAMPLERS

APPENDIX N

COLLECTION EFFICIENCY OF LITTON MODEL M LARGE VOLUME SAMPLERS

The Litton Model M large volume sampler (LVS), used to collect aerosol data, is an electrostatic precipitator. During operation of an LVS, an electrical potential of approximately 15,000 volts (15 kV) is maintained across an 11/16-inch spacing between the plate and disk. This creates two effects: 1) a corona is emitted from a ring of 60 needles thereby giving the microorganism particles a charge and 2) the resultant electric field attracts the charged particles to the collecting disk.

Collection efficiencies for electrostatic precipitators depend on the operating high voltage producing the internal charging corona and electric field. Sufficient voltage must be supplied to the corona source to charge the particles suspended in air; the greater the voltage, the greater the driving force to effect particle separation from air.

Electrostatic precipitators are usually operated at the highest voltage possible without sparking (arcing). Sparking disrupts the operation of the electrical equipment and lowers collection efficiency by reducing the applied voltage, redispersing the collected particles, and promoting current channeling (effectively reducing particle charging and collection to localized areas).

Very high dust loadings increase the potential difference required for the production of a corona and reduce the current due to the space charge of the particles. This tends to reduce the average particle charge and reduces collection efficiency. Compensation can be obtained by increasing the potential difference when high dust loadings are involved.

The collection efficiency of an LVS is affected by many other factors than simply the operating voltage and dust loading. The performance will change according to intake air velocity, particle size distribution, particle concentration in air, and environmental conditions (e.g., wind gusts, wind speed, direction, and relative humidity).

Data obtained from field operation of the LVS are used in LHES to calculate microbial concentrations in air as discussed in the second annual LHES report (Calculation of Microorganism Density in Air section). The resultant microbial concentrations assist the interpretation of the degree of aerosol exposure an individual would receive based upon the time of day and distance from an operating rig whose source is either reservoir or pipeline wastewater. Thus, it is important to correct all LVS sampling data to a reference set of operating conditions to obtain internally consistent data. For example, an LVS may measure 20 cfu/m^3 of air with operating conditions which result in a relative collection efficiency of 40% and its paired sampler may measure 40 cfu/m^3 of air with different operating conditions which have a relative collection efficiency of 80%. If only the raw data were used to calculate microbial concentrations without regards to operating conditions (i.e., collection efficiency), then one would incorrectly conclude that the second sampler observed microbial concentrations twice as great as the first sampler. If the reference set of operating conditions had an effective collection efficiency of 100%, then both samplers would be recorded as having measured 50 cfu/m^3 of air.

To determine correction factors for operating conditions, rigorous experimentation is required in a controlled environment. A few environmental conditions can be reconstructed in the laboratory to evaluate their effect on collection efficiency. However, certain factors such as microbial concentrations, particle size, and wind gusts cannot be evaluated. Thus, the calculated microbial concentrations will be subjected to indeterminate errors; the magnitude of these errors cannot be estimated. Some factors (e.g., operating voltage) are known to affect the collection efficiency and since these can be evaluated, it is necessary to adjust the raw data for these factors.

The Naval Biosciences Laboratory (NBL) in Oakland, California conducted experiments on three separate occasions (1976, July 1980 and October 1982) to develop a collection efficiency data base from which to calculate correction factors. In all of the NBL studies, data were obtained for relative collection efficiencies of LVS to all-glass impingers (AGI) samplers in a controlled environment (an atomizer created a specified amount of aerosol in an enclosed wind tunnel). In these studies the AGI samplers had a high degree of precision (for November 1982 NBL data the average $s/\bar{x}$ was 6.70%), but their accuracy was not evaluated. On the other hand, the LVS performed with less precision, as is demonstrated by the average $s/\bar{x}$ of 11.7% for operating voltages greater than 12 kV (precision decreases for smaller operating voltages).

The experimental procedures employed by NBL to study the LVS collection efficiencies are thoroughly documented in their three final reports; a capsule summary of these reports follows.

Disinfecting procedures prior to a sampling period were identical for all three NBL studies and SwRI field operations. Operating time for the samplers varied in each study, but discrepancies among the reported results should not be caused by this procedural change since the results are reported as relative collection efficiencies (relative to AGI samplers operating simultaneously with the LVS in the same wind tunnel).

Bacillus subtilis var. Niger replaced Flavobacterium as the test organism for the November 1982 NBL study. Bacillus subtilis var. Niger is a hardy spore, but in spite of this, no problems of residual contamination carryover were encountered.

Before the 1976 study, the samplers were completely overhauled; defective and worn parts were either replaced or repaired. For the other two NBL studies, the samplers were not overhauled; however, routine preventative maintenance was continued. It is unknown whether the 1976 overhaul affected collection efficiencies differently than the routine maintenance procedures.

The collection fluid (BHI) circulation rate varied among all three NBL studies. In July 1980, NBL reported that no collection efficiency differences were observed for a BHI rate greater than 8 mL/min; only data obtained with BHI rates greater than this were used for correction factor evaluations. The air intake sampling rates were approximately 1.0 m^3/min.

LVS operating voltages in the three NBL studies ranged from 8 to 18 kV. The 1976 study reported two LVS sampler responses at various operating voltages (8 to 14 kV) were obtained by NBL for LVS samplers operated at the highest voltage attainable without producing excessive arcing; these data are reported as relative collection efficiencies at 15+ kV. The October 1982 data is reported on raw data sheets as relative collection efficiencies at the actual LVS operating voltage.

During the effort to identify operating variables that influence the LVS collection efficiency, NBL studied relative humidity and temperature effects in the October 1982 study. According to NBL no strong effect of relative humidity was observed for the range tested (relative humidities from 51 to 81), and the rather narrow temperature range (unqualified) of the tests showed no collection efficiency effects. These conclusions from NBL are most likely incomplete for two reasons:

1) When relative humidity is plotted versus collection efficiency a negative correlation between collection efficiency and relative humidity for voltages greater than 12 kV is suggested (see Figure N.1). This correlation is less apparent for operating voltages of 12 kV. Insufficient data makes it impossible to evaluate the effect of relative humidity at lower operating voltages.

2) NBL does not report the operating temperatures, but it seems unlikely that the wide temperature range in the field (10 to 35°C) was adequately studied.

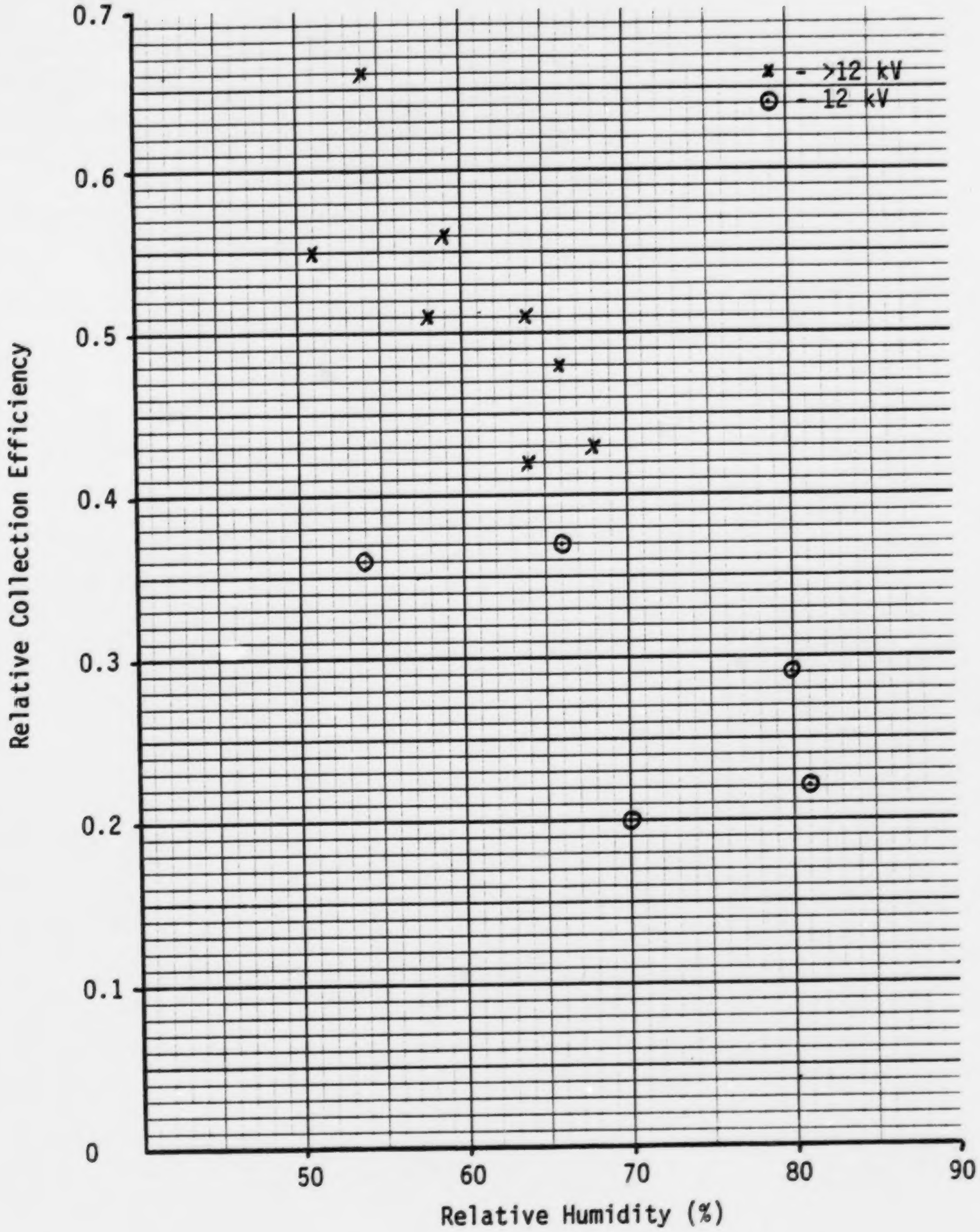

Figure N.1. Relative humidity versus relative collection efficiency (LVS/AGI ratio) (1982 NBL data)

Nevertheless, correction factors for temperature and relative humidity are not applied to field data since insufficient data exist to formulate accurate correction factors.

Only the October 1982 NBL report included data that could be used to develop an air flow rate correction factor C_f. Each sampler will require a unique C_f; however, NBL only reported one data value for each LVS. Since neither reproducibility nor accuracy was demonstrated by NBL for C_f, it is not recommended to use the "correction factors" to adjust field data; it is presumed that exclusion of C_f will not result in severe deficiencies in the final evaluation since none of these corrections changed the raw data by more than 20%.

It would be possible to obtain sufficient data to derive a reasonable correction factor by repeating these tests for each sampler set at the 1000 L/min mark. However, these data experiments are unwarranted, since during field sampling wind gusts alter the sampling air flow rate making it impossible to achieve the same laboratory precision in determining the air flow rate.

In October 1982 NBL measured the voltage supplied to the corona source at four different high voltage settings on nine different LVS samplers. Calibration curves were drawn for each sampler by plotting the indicated versus the measured voltage. Each calibration curve was a straight line with a slope of approximately one but with various y-intercepts. Repetition of the voltage measurements was not reported, so it is unknown whether these results are reproducible. Consequently, the voltage correction factor uses the recorded operating voltage as the independent variable, not the actual measured voltage.

To determine whether each LVS should have an individual sampler correction factor, the 1982 NBL data was analyzed at SwRI on a Cyber 170/171 with the SPSS package and the ANOVA subroutine. No consistent differences were observed among samplers both at the 12 kV and at greater than 12 kV (15+ kV). It appeared that the actual run numbers had greater significance than individual samplers. The significance may be partly due to relative humidity values; other operating variables (e.g., temperature) may also contribute to the difference observed between runs.

In the 1976 NBL study, no effects from operating at voltages greater than 12 kV were observed. However, in the October 1982 study, large variations of collection efficiencies occur for LVS operating at voltages greater than 12 kV. At this time there is no explanation for these conflicting results.

The raw data from the October 1982 NBL study are plotted on a semilog plot in Figure N.2 (operating voltage versus relative collection efficiencies of LVS to AGI samplers). From these data, four different correction factor curves could be drawn.

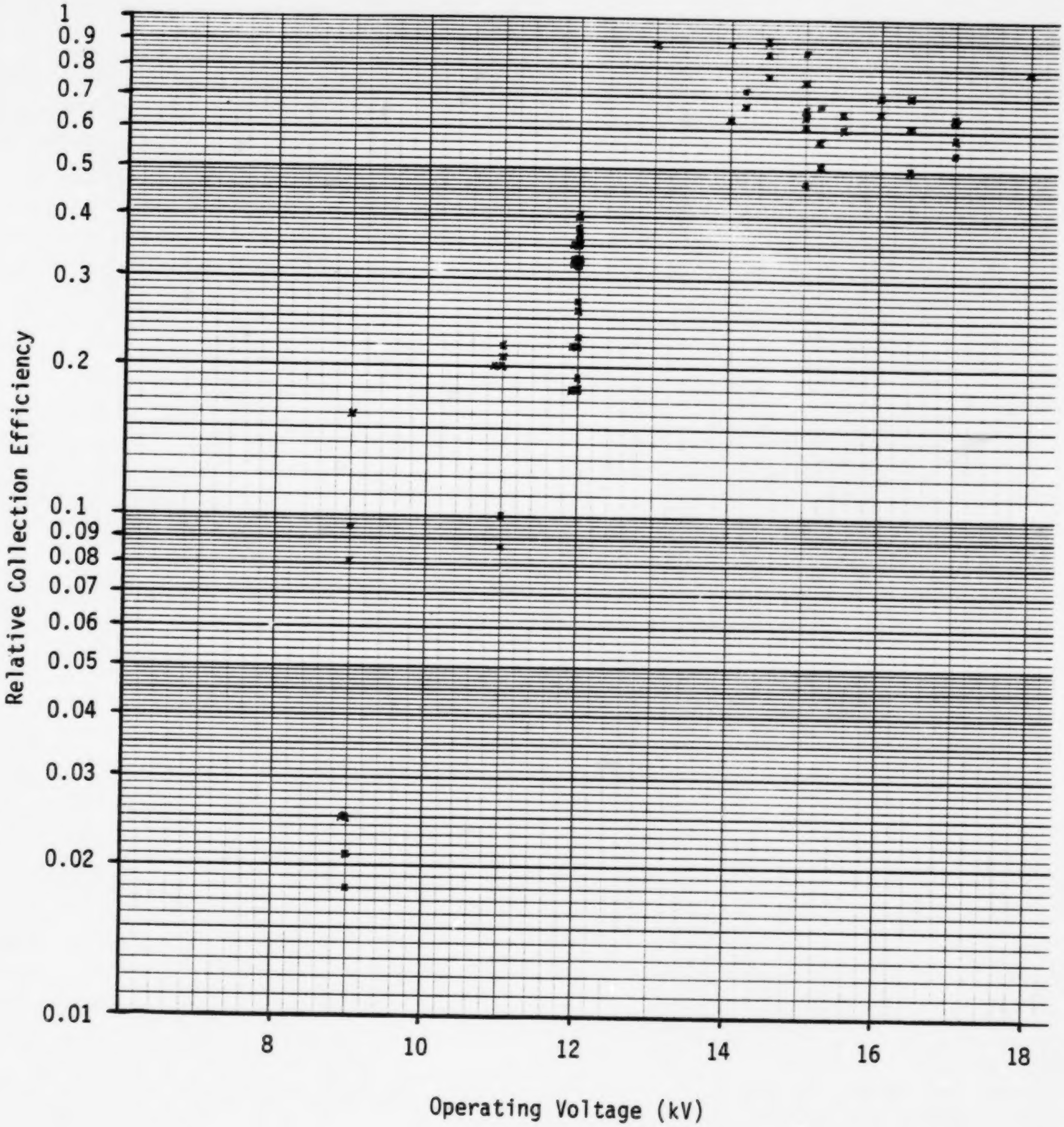

Figure N.2. Operating voltage versus relative collection efficiency (LVS/AGI ratio) (1982 NBL data)

In the field, several measurements were made with paired samplers. These paired field samplers may help to identify the most valid correction factor, i.e., the correction factor that minimizes the difference between the reported microbial concentrations for all microorganisms for all paired samplers.

The four possible correction factor curves are plotted in Figure N.3. Curve A represents no correction factor. Curve B is modeled after the 1976 data where data below 12 kV are corrected as an average between reported values and above 12 kV no correction is made. The third method (Curve C) was calculated from all averages at various voltages from the 1982 NBL data. Curve D is a minimum correction factor.

The physical interpretation for Curve A is that an LVS sampler operates similarly at all voltages. From the preceding discussion, it is known that this is unrealistic.

Curve B assumes that once the operating voltage reaches 12 kV no effect on collection efficiency is observed as long as operation occurs below sparking. In addition, this correction factor has no minimum asymptote for operating voltages below 12 kV.

The third correction factor (Curve C) demonstrates the same low voltage correction as Curve B. High voltage operation distinguishes between these two methods. In Curve C the NBL 1982 data is corrected to 12 kV. Since the data peaks at 14 kV, an inflection point is observed at 11.5 kV, a maximum at 14 kV, and then an asymptote at 14.5 kV. No minimum asymptote exists. A physical interpretation could be the following: at low voltages, the collection efficiency increases proportionately with the operating voltage. At 11.5 kV all of the particles are charged. Greater voltages affect a greater driving force for separating the charged particles from the air. Above 14 kV visually undetectable sparking occurs that reduces the effective voltage until it reaches an asymptote in which the increased sparking is counteracted by the increased driving force from the high voltage.

Curve D has a minimum asymptote that implies that under certain field conditions, a low voltage will always be able to charge a few particles and will be able to collect these. Moving from the asymptote, at higher operating voltages, proportionately more particles are charged and consequently collected. At 11.5 kV, an inflection point occurs that implies that a different mechanism is responsible for greater collection efficiencies. It is hypothesized that at 11.5 kV all particles are charged but that the collecting electric field determines the percentage of particles that are collected. Thus, an increase in operating voltage above 11.5 kV increases the electric field which in turn increases the collecting

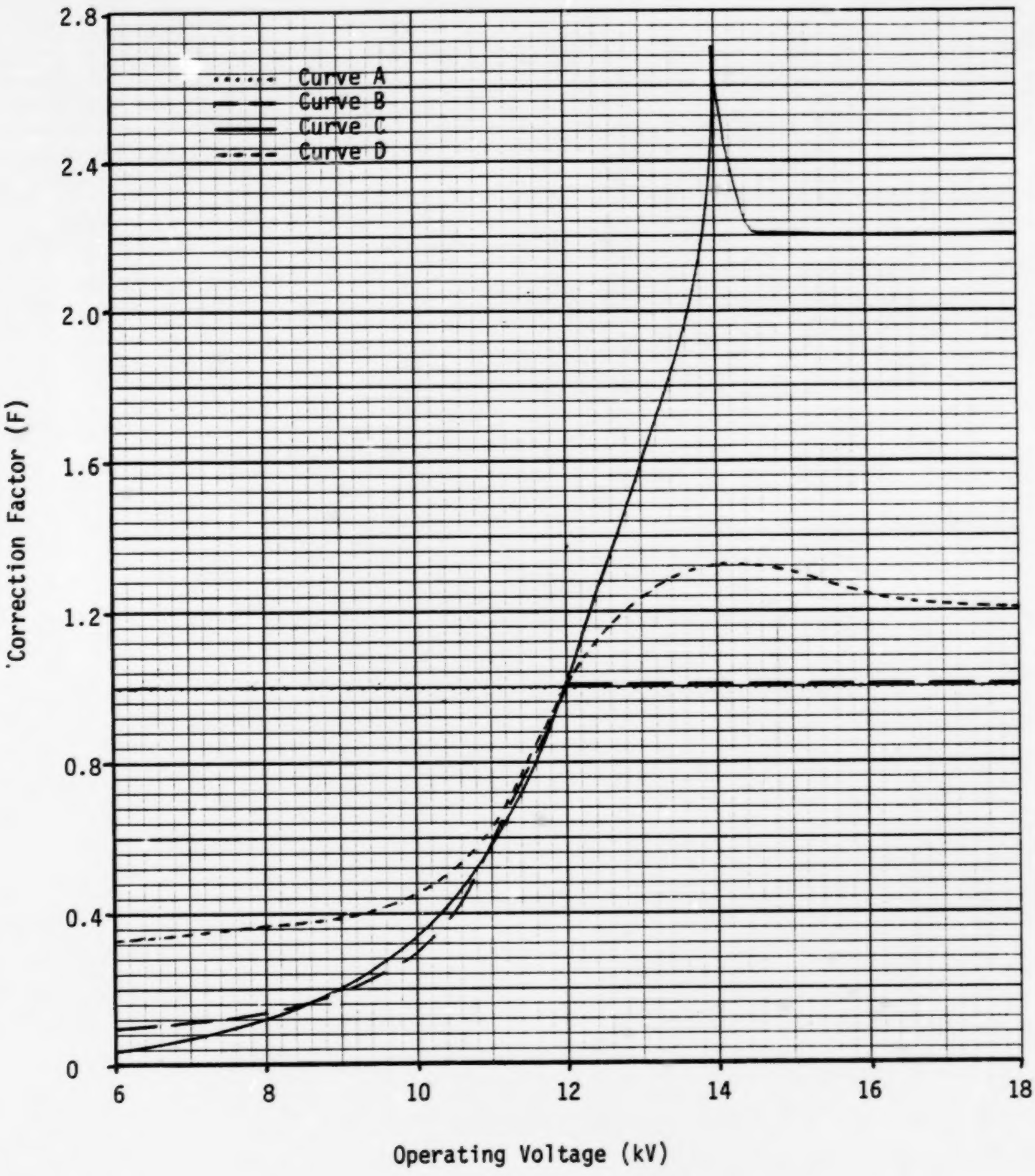

Figure N .3. Comparison of four alternative correction factor curves driving force. A maximum asymptote is then observed where an increase in operating voltage increases the sparking phenomenon which reduces the effective electric field. If operation had occurred while excessive sparking occurred, it is predicted that Curve D would show a decrease in collection efficiency beyond the maximum asymptote.

Differences in Curves C and D are a result of the calculational basis of correction. Curve C was calculated from average efficiencies at various operating voltages; Curve D was calculated from the highest efficiency observed for operating voltages below 12 kV and lowest efficiencies observed for voltages above 12 kV. The latter produces a conservative correction that adjusts all data to the minimal degree expected. Thus, the corrected data may be required to be adjusted further, but it will never be overcorrected.

Curve D seems to be more realistic than Curves A and B because the data suggest that some correction is required in both the high and low voltage regions. It also seems to be more realistic than Curve C because it will not result in overcorrections. This latter is an important consideration since at low voltages (9 kV) an order of magnitude range was observed in the experimental data (see Figure N.2).

All of the field data are corrected using Curve D (minimum corrections) and are presented in the aerosol data results section along with a table of all of the operating field voltages. The correction factors employed are in Table 4.15 of the Calculation of Microorganism Density in Air section. With these data, the interested reader can develop his own correction factor method and test it against the field data (paired samples).

APPENDIX O

ENTEROVIRUS SEROLOGY QUALITY CONTROL:
TITER REPRODUCIBILITY (TR) FROM REPLICATE TESTING

VIRUS: Adenovirus 3

TITER	FREQUENCY DISTRIBUTION High	FREQUENCY DISTRIBUTION Intermediate
< 10		
10	1	
20		6
40	4	12
80	8	
160	5	
320		
≥ 640		
TOTAL	18	18
GEOMETRIC MEAN TITER	91	32
TR	0.79	1.00

SOURCE OF CONTROL SERA:

High titer 605

Intermediate titer 804

RUN #	DATE	VIRUS DOSE $(TCID_{50})$	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	7-11-83	41	141	1.00	32	1.00
2	11-16-83	316	50	1.00	32	1.00
3	2-02-84	147	56	1.00	32	1.00

VIRUS: Adenovirus 5

SOURCE OF CONTROL SERA:

High titer 504

Intermediate titer 704

TITER	FREQUENCY DISTRIBUTION High	Intermediate
< 10		
10		
20		1
40	3	15
80	3	7
160	9	
320	6	
≥ 640		
TOTAL	21	23
GEOMETRIC MEAN TITER	145	50
TR	0.71	0.97

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	7-06-83	261	40	1.00	40	1.00
2	11-17-83	178	224	0.78	50	1.00
3	2-02-84	178	125	1.00	56	1.00
4	3-01-84	178	202	1.00	45	0.88

VIRUS: Adenovirus 7

SOURCE OF CONTROL SERA:

High titer 707

Intermediate titer 704

	FREQUENCY DISTRIBUTION	
TITER	High	Intermediate
< 10		7
10	1	11
20	21	19
40	16	1
80		
160		
320		
≥ 640		
TOTAL	38	38
GEOMETRIC MEAN TITER	26	13
TR	0.97	0.89

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	10-06-82	178	18	1.00	9	1.00
2	10-07-82	178	22	1.00	13	1.00
3	10-19-82	178	22	1.00	18	1.00
4	11-17-83	316	28	1.00	5	1.00
5	2-02-84	100	40	1.00	22	1.00
6	3-01-84	215	36	1.00	18	1.00

VIRUS: Coxsackie B2

TITER	FREQUENCY DISTRIBUTION High	 Intermediate
< 10		
10		
20		
40		2
80		12
160		5
320	1	
≥ 640	16	
TOTAL	17	19
GEOMETRIC MEAN TITER	614	89
TR	1.00	0.94

SOURCE OF CONTROL SERA:

High titer 802

Intermediate titer 610

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	5-18-83	40	640	1.00	100	1.00
2	5-20-83	26	640	1.00	112	1.00
3	2-28-84	41	557	1.00	65	1.00

VIRUS: Coxsackie B4

SOURCE OF CONTROL SERA:

High titer 601

Intermediate titer 800

TITER	FREQUENCY DISTRIBUTION High	 Intermediate
< 10		
10	1	5
20	6	7
40	10	4
80	1	2
160		
320		
≥ 640		
TOTAL	18	18
GEOMETRIC MEAN TITER	31	22
TR	0.95	0.86

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	8-01-83	100	32	1.00	20	0.95
2	8-02-83	164	20	0.95	13	1.00
3	1-19-84	83	45	0.83	45	0.89

VIRUS: Coxsackie B5

	FREQUENCY DISTRIBUTION	
TITER	High	Intermediate
< 10		4
10		12
20		26
40		7
80	14	1
160	18	
320	7	
≥ 640	5	
TOTAL	44	50
GEOMETRIC MEAN TITER	168	17
TR	0.87	0.82

SOURCE OF CONTROL SERA:

High titer 904

Intermediate titer 713

RUN #	DATE	VIRUS DOSE $(TCID_{50})$	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	2-03-82	316	224	0.56	16	0.83
2	2-04-82	144	457	0.78	13	0.83
3	10-05-82	68	132	0.88	18	1.00
4	5-04-83	56	174	0.81	22	1.00
5	11-02-83	32	141	1.00	14	1.00
6	1-17-84	178	100	1.00	18	1.00
7	2-28-84	121	141	0.89	45	1.00

VIRUS: Echovirus 1

SOURCE OF CONTROL SERA:

High titer 42502

Intermediate titer 32401

TITER	FREQUENCY DISTRIBUTION High	FREQUENCY DISTRIBUTION Intermediate
< 10		1
10		3
20		7
40		1
80	6	
160	6	
320		
≥ 640		
TOTAL	12	12
GEOMETRIC MEAN TITER	113	16
TR	1.00	0.92

RUN #	DATE	VIRUS DOSE (TCID$_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	11-18-82	32	---	---	---	---
2	11-19-82	32	---	---	---	---
3	10-27-83	24	112	1.00	13	0.83
4	1-31-84	122	112	1.00	20	0.94

VIRUS: Echovirus 3

TITER	FREQUENCY DISTRIBUTION High	FREQUENCY DISTRIBUTION Intermediate
< 10		
10		10
20	2	18
40	6	5
80	13	7
160	9	
320	5	
≥ 640		
TOTAL	35	40
GEOMETRIC MEAN TITER	96	23
TR	0.71	0.69

SOURCE OF CONTROL SERA:

High titer 802

Intermediate titer 601

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	5-25-83	242	45	0.67	11	1.00
2	5-27-83	242	71	1.00	18	1.00
3	11-12-83	32	105	0.68	14	1.00
4	1-18-84	48	200	0.83	22	1.00
5	2-16-84	10	141	0.89	56	0.78
6	2-21-84	≤ 10	cont.	cont.	50	0.83 cont.
7	2-23-84	32	79	0.77	25	0.83

VIRUS: Echovirus 5

SOURCE OF CONTROL SERA:

High titer 42702

Intermediate titer 41001

	FREQUENCY DISTRIBUTION	
TITER	High	Intermediate
< 10		1
10		
20	1	2
40	4	7
80	2	9
160	5	
320	7	
≥ 640	1	
TOTAL	20	19
GEOMETRIC MEAN TITER	139	46
TR	0.56	0.81

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	3-10-82	100	20^{*a}	1.00^{*}	5^{*a}	1.00^{*a}
2	3-11-82	215	32^{*a}	1.00^{*a}	5^{*a}	1.00^{*}
3	10-04-82	261	209	0.88	74	1.00
4	10-27-83	64	40	0.94	25	0.78
5	2-24-84	32	282	0.89	46	0.88

[a] Due to the low titers in staff sera, study participant sera was used as controls in runs 3-5; the staff titers were not included in the frequency distribution.

VIRUS: Echovirus 9

SOURCE OF CONTROL SERA:

High titer 702

Intermediate titer 709

TITER	FREQUENCY DISTRIBUTION High	Intermediate
< 10		4
10		17
20		15
40	5	
80	18	
160	13	
320	3	
≥ 640		
TOTAL	39	36
GEOMETRIC MEAN TITER	103	12
TR	0.82	0.91

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	3-03-82	68	200	1.00	18	1.00
2	3-04-82	56	100	0.83	13	0.83
3	10-21-82	178	74	1.00	9	1.00
4	11-03-83	32	126	0.67	18	1.00
5	2-02-82	100	112	1.00	13	1.00

VIRUS: Echovirus 11

SOURCE OF CONTROL SERA:

High titer 32111

Intermediate titer 22411

TITER	FREQUENCY DISTRIBUTION High	FREQUENCY DISTRIBUTION Intermediate
< 10		
10		9
20	2	19
40	12	6
80	17	2
160	4	
320	2	
≥ 640		
TOTAL	37	36
GEOMETRIC MEAN TITER	69	20
TR	0.78	0.83

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	3-10-82	178	10*[a]	1.00	5	1.00
2	3-11-82	147	13*[a]	1.00	5	1.00
3	10-20-82	316	79	0.97	15	1.00
4	11-03-83	83	56	1.00	14	1.00
5	1-12-84	68	63	0.61	45	0.83
6	2-09-84	75	40	0.92	14	1.00
7	2-10-84	100	*b		*b	
8	2-14-84	53	143	0.67	28	1.00
9	2-28-84	100	56	1.00	28	1.00

[a] Due to low titers in the staff sera, study participant sera was used as controls in runs 3-9.

[b] Control titers for this run were misplaced.

VIRUS: Echovirus 17

SOURCE OF CONTROL SERA:

High titer 800

Intermediate titer 614

TITER	FREQUENCY DISTRIBUTION High	FREQUENCY DISTRIBUTION Intermediate
< 10		2
10		8
20	11	18
40	9	2
80	8	
160	1	
320		
≥ 640		
TOTAL	29	30
GEOMETRIC MEAN TITER	39	13
TR	0.87	0.88

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	6-01-83	241	25	1.00	11	0.88
2	6-03-83	562	28	1.00	16	1.00
3	11-09-83	242	25	1.00	14	0.78
4	1-19-84	133	89	1.00	25	1.00
5	2-28-84	56	50	0.83	16	1.00

VIRUS: Echovirus 19

SOURCE OF CONTROL SERA:
High titer 702
Intermediate titer 704

TITER	FREQUENCY DISTRIBUTION High	FREQUENCY DISTRIBUTION Intermediate
< 10		5
10	3	4
20	2	8
40	9	
80	2	
160		
320		
≥ 640		
TOTAL	16	17
GEOMETRIC MEAN TITER	31	11
TR	0.74	0.86

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	7-05-83	61	45	1.00	19	1.00
2	11-10-83	100	13	1.00	5	1.00
3	1-17-84	130	50	1.00	14	1.00

VIRUS: Echovirus 20

SOURCE OF CONTROL SERA:

High titer 702

Intermediate titer 614

	FREQUENCY DISTRIBUTION	
TITER	High	Intermediate
< 10		2
10	5	5
20	11	11
40	4	4
80	3	
160		
320		
≥ 640		
TOTAL	23	22
GEOMETRIC MEAN TITER	23	13
TR	0.87	0.89

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	8-09-83	83	20	0.94	22	0.89
2	8-10-83	130	16	0.83	16	1.00
3	11-10-83	100	18	1.00	7	1.00
4	2-28-84	56	56	1.00	25	1.00

VIRUS: Echovirus 24

	FREQUENCY DISTRIBUTION	
TITER	High	Intermediate
< 10		8
10		6
20	1	3
40	2	2
80	5	4
160	7	1
320	5	
≥ 640	1	
TOTAL	21	24
GEOMETRIC MEAN TITER	135	15
TR	0.58	0.58

SOURCE OF CONTROL SERA:

High titer 904

Intermediate titer 702

RUN #	DATE	VIRUS DOSE ($TCID_{50}$)	GEOM. MEAN High Titer	TR High Titer	GEOM. MEAN Interm. Titer	TR Interm. Titer
1	7-26-83	75	112	1.00	8	0.83
2	7-28-83	90	112	1.00	14	0.72
3	11-09-83	56	63	0.72	6	1.00
4	1-31-84	32	320	0.89	80	0.89

APPENDIX P

SUPPLEMENTAL TABLES FOR SECTION 5 (RESULTS)

TABLE P-1. MICROORGANISM CONCENTRATIONS IN LUBBOCK WASTEWATER

24-Hour composite samples analyzed	Sampling date						
	1980			1981			
	Jun 3-4	Jul 28-29	Nov 3-4	Jan 19-20	Feb 16-17	Mar 9-10	Mar 23-24
Bacteria (cfu/mL)							
Standard plate count	3,600,000	5,700,000	3,400,000				
Total coliforms	350,000	380,000	140,000	60,000	110,000	120,000	160,000
Fecal coliforms	87,000	72,000	88,000	15,000	34,000	16,000	83,000
Fecal streptococci	4,700	2,000	5,100				
Mycobacteria sp.	1,200	170,000	1,100				
Clostridium perfringens[a]							
- vegetative	7,500	110,000	2,400				
- sporulated	930	430	930				
Staphylococcus aureus	<33	<3	<3				<10
Salmonella sp.	<0.004	≥0.002	<0.002				<0.01[b]
Shigella sp.	<0.004	≤0.002	<0.002				≥0.01
Yersinia enterocolitica	<0.002	<0.004	<0.004				100
Campylobacter jejuni							88
Candida albicans							<0.3
Fluorescent Pseudomonas sp.	10,000	6,300	3,100				
Klebsiella sp.	<33,000	130,000	53,000				130,000
Viruses (pfu/mL)							
Bacteriophage	1,400	3,200	2,600	880			
Enteroviruses							
HeLa, 5 day (uncorrected)	0.78	1.2	0.73	0.086	0.054	0.059	0.046
HeLa, polio-neutralized							
RD, polio-neutralized							
Poliovirus concentration efficiency (%)	38	42	39	97	78	28	105
Physical Analyses (mg/L)							
Total organic carbon	83	40		115	133	141	91
Total suspended solids	98	78	215	184	151	234	89
Total volatile suspended solids	65	52	135	130	120	178	74
pH	6.5	6.8	7.2	7.0	7.3	7.0	7.1

continued...

TABLE P-1. (CONT'D)

24-Hour composite samples analyzed	Sampling date						
	1981						
	Apr 20-21	May 4-5	Jun 15-16	Jun 29-30	Jul 20-21	Aug 17-18	Nov 17-18
Bacteria (cfu/mL)							
Standard plate count	9,600,000				3,000,000		
Total coliforms	520,000		360,000	120,000	380,000		
Fecal coliforms	59,000	86,000	110,000	50,000	100,000	91,000	60,000
Fecal streptococci	6,900		1,100	8,700	2,400		
Mycobacteria sp.	400,000				14,000		
Clostridium perfringens[a]							
- vegetative	110,000				230		
- sporulated	460				210		
Staphylococcus aureus	3	<3	<3	<3	<10	<3	3
Salmonella sp.	≥0.006	≥0.005	<0.01	<0.01	≥10	≥10	≥10
Shigella sp.	≥0.008	≥1	<0.01	<0.01	<0.007	<0.008	<0.01
Yersinia enterocolitica	<0.006	≥0.005	<0.01	<0.01	<0.007	<0.008	<0.01
Campylobacter jejuni		<3	<3	≥200[c]	<10	<0.1	<3
Candida albicans	<3	<3	<3	<3	<10	<3	<3
Fluorescent Pseudomonas sp.	220,000				23,000		
Klebsiella sp.	230,000	2,600	200,000	30,000	86,000	50,000	130,000
Viruses (pfu/mL)							
Bacteriophage	1,600				2,100		
Enteroviruses							
HeLa, 5 day (uncorrected)	0.067	0.11	0.1	0.086	0.065	0.045	0.055
HeLa, polio-neutralized	0.018	0.006	0.066	0.065	0.02	0.005	0.0013
RD, polio-neutralized	0.008	0.033	0.15	0.1	0.083	0.42	0.13
Poliovirus concentration efficiency (%)	88	95	79	77	85	34	80
Physical Analyses (mg/L)							
Total organic carbon	237	104	47	100	100	78	100
Total suspended solids	200	115	47	51	43	68	118
Total volatile suspended solids	147	92	44	36	33	49	87
pH	7.5	7.8	6.5	7.8	7.2	6.4	7.3

continued...

TABLE P-1. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1982						
	Feb 15-16^d	Feb 16^{d,e}	Mar 1-2	Mar 8-9^a	Mar 15-16	Mar 22-23	Mar 29-30
Bacteria (cfu/mL)							
Standard plate count		150					
Total coliforms		240				57,000	
Fecal coliforms	11,000	38	5,600	75,000	79,000	81,000	50,000
Fecal streptococci	11,000	120	1,000	5,900	3,500	7,900	5,000
Mycobacteria sp.		1,000	28,000	53,000	30,000	13,000	10,000
Clostridium perfringens^e							
- vegetative		210					
- sporulated		28					
Staphylococcus aureus		<3	2.5	<3		<3	
Salmonella sp.		<0.04	≥0.04			100	
Shigella sp.		<0.01	<0.01	<0.01		<0.01	
Yersinia enterocolitica		<0.01	<0.01	<0.01		<0.01	
Campylobacter jejuni		<0.01	<3	40		<3	
Candida albicans		<3	<3	<3		<3	
Fluorescent Pseudomonas sp.		30				280,000	
Klebsiella sp.	130,000	180	50,000	68,000		50,000	
Viruses (pfu/mL)							
Bacteriophage	900	750	1,000	1,800	780	1,500	69
Enteroviruses							
HeLa, 5 day (uncorrected)	0.037	0.033	0.07	0.11	0.11	0.063	0.012
HeLa, polio-neutralized	<0.003	<0.005	0.034	0.022	0.017	0.004	0.002
RD, polio-neutralized	<0.003	<0.002	<0.002	<0.002		0.010	0.034
Poliovirus concentration efficiency (%)	227		50	86	f	63	
Physical Analyses (mg/L)							
Total organic carbon	136	103	98	116	95	151	125
Total suspended solids	111	143	150	178	92	269	205
Total volatile suspended solids	96	90	113	153	82	170	165
pH	7.1	8.6	7.1	7.1	7.4	7.3	7.1

continued...

TABLE P-1. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1982						
	Apr 5-6	Apr 19-20	Apr 26-27[g]	Jun 14-15	Jun 29-30	Jul 26-27	Aug 9-10
Bacteria (cfu/mL)							
Standard plate count						1,300,000	
Total coliforms						120,000	
Fecal coliforms	84,000	110,000	9,100	66,000	68,000	58,000	35,000
Fecal streptococci	2,800	4,800	1,800	1,000	4,200	2,300	2,500
Mycobacteria sp.	20,000	6,000	8,500	13,000	43,000	13,000	
Clostridium perfringens[a]							
- vegetative						750	
- sporulated						9	
Staphylococcus aureus	<3					<3	
Salmonella sp.	0.01	≥1.0			≥1.0	≥0.1	≥1
Shigella sp.	<0.01	<0.01			<0.01	<0.01	<0.01
Yersinia enterocolitica	1,000	100			<0.01	<0.01	<0.01
Campylobacter jejuni	<3	10			<10	10	<3
Candida albicans	<3	40			<10	<3	<3
Fluorescent Pseudomonas sp.					9,000[h]	6,000	730
Klebsiella sp.	1,000	130,000			100,000	5,000	26,000
Viruses (pfu/mL)							
Bacteriophage	380	830	220	840	840	1,100	
Enteroviruses							
HeLa, 5 day (uncorrected)	0.017	0.042	0.028	0.026	0.49	0.060	0.087
HeLa, polio-neutralized	0.004	0.016	0.008	0.026	0.39	0.030	0.074
RD, polio-neutralized	0.044	0.010	0.004	<0.002	0.056	0.007	2.2
Poliovirus concentration efficiency (%)	77	54	69	64	68	163	156
Physical Analyses (mg/L)							
Total organic carbon	102	71	98	72	59	69	67
Total suspended solids	151	118	98	77	111	140	105
Total volatile suspended solids	119	96	79	66	84	106	74
pH	7.8	7.6	7.5	7.2	7.3	7.5	7.6

continued...

TABLE P-1. (CONT'D)

24-Hour composite samples analyzed	Sampling date						
	1982				1983		
	Aug 30-31[e]	Sep 13-14	Nov 1-2	Dec 13-14	Feb 16-17	Mar 7-8	Mar 21-22
Bacteria (cfu/mL)							
Standard plate count							
Total coliforms			210,000	170,000	140,000	230,000	330,000
Fecal coliforms	200	65,000	48,000	31,000	58,000	23,000	6,100
Fecal streptococci	30	3,500	2,100	900	3,000	8,000	4,000
Mycobacteria sp.	760	1,400					
Clostridium perfringens[j]							
- vegetative							k
- sporulated							10
Staphylococcus aureus							
Salmonella sp.	<0.01	≥0.01	-	+	+	-	+
Shigella sp.[m]	<0.01	<0.01					<0.01
Yersinia enterocolitica[m]	<0.01	<0.01					≥0.01
Campylobacter jejuni	<3	≥10					
Candida albicans	<3	<300					
Fluorescent Pseudomonas sp.[n]	30,000	2,000					<0.33
Klebsiella sp.	300	40,000					2,000
Viruses (pfu/mL)							
Bacteriophage	30						5,500
Enteroviruses							
HeLa, 5 day (uncorrected)	1	0.022	0.11	0.092	0.044	0.11	0.031
HeLa, polio-neutralized	1	0.008	0.092	0.018	0.020	0.012	0.018
RD, polio-neutralized	0.018	0.84	0.52	0.082	0.028	0.072	0.024
Poliovirus concentration efficiency (%)	47	42	o	o	16	92	69
Physical Analyses (mg/L)							
Total organic carbon	52	58	54	o	49	109	83
Total suspended solids	51	50	91	o	78	126	83
Total volatile suspended solids	39	42	69	o	55	102	68
pH	7.3	7.8	7.3	7.7	7.7	7.6	7.5

continued...

TABLE P-1. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1983						
	Apr 4-5	Apr 18-19	Jun 27-28	Jul 11-12	Jul 25-26	Aug 8-9	Aug 22-23
Bacteria (cfu/mL)							
Standard plate count							
Total coliforms	160,000	140,000					
Fecal coliforms	20,000	18,000	59,000	53,000	48,000	120,000	90,000
Fecal streptococci	3,100	4,000	1,200	1,200	500	1,000	0.2
Mycobacteria sp.							
Clostridium perfringens[j]							
- vegetative		<5.0[l]					
- sporulated		<5.0[l]					
Staphylococcus aureus							
Salmonella sp.	-	-	-	+	+	+	+
Shigella sp.[m]		<0.01					
Yersinia enterocolitica[m]		<0.01					
Campylobacter jejuni							
Candida albicans							
Fluorescent Pseudomonas sp.[n]		400					
Klebsiella sp.		100					
Viruses (pfu/mL)							
Bacteriophage		4,000					
Enteroviruses							
HeLa, 5 day (uncorrected)	0.12	0.10	0.27	0.26	0.29	0.12	0.24
HeLa, polio-neutralized	0.044	<0.004	0.14	0.30	0.12	0.13	0.38
RD, polio-neutralized	0.060	<0.004	0.34	0.68	0.16	0.18	0.20
Poliovirus concentration efficiency (%)	47	88	72	44	42	30	61
Physical Analyses (mg/L)							
Total organic carbon	62	50	42	35	22	28	32
Total suspended solids	58	41	35	23	29	44	17
Total volatile suspended solids	51	31	25	16	23	34	14
pH	7.6	7.8	7.6	7.7	7.6	7.1	7.9

continued...

TABLE P-1. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1983 Sep 12-13
Bacteria (cfu/mL)	
Standard plate count	
Total coliforms	
Fecal coliforms	210,000
Fecal streptococci	1,000
Mycobacteria sp.	
Clostridium perfringens[j]	
- vegetative	
- sporulated	
Staphylococcus aureus	
Salmonella sp.	-
Shigella sp.[m]	
Yersinia enterocolitica[m]	
Campylobacter jejuni	
Candida albicans	
Fluorescent Pseudomonas sp.[n]	
Klebsiella sp.	
Viruses (pfu/mL)	
Bacteriophage	
Enteroviruses	
HeLa, 5 day (uncorrected)	0.056
HeLa, polio-neutralized	0.18
RD, polio-neutralized	0.12
Poliovirus concentration efficiency (%)	74
Physical Analyses (mg/L)	
Total organic carbon	37
Total suspended solids	25
Total volatile suspended solids	17
pH	7.3

a. Most probable number (MPN)/mL.
b. A new procedure was used for detection of Salmonella spp. (Kaper et al., App. Environ. Microbiol., 33:829-35, 1977) beginning in March 1981.
c. Value calculated from representative colonies identified as C. jejuni, actual number may be higher.
d. On February 16, 1982 the sample source was changed from the trickling filter to the pipeline; the first set of data on February 16 was sampled from the trickling filter while the second set was collected from the pipeline.
e. Chlorination of wastewater at treatment plant.
f. Lost.
g. Chlorination in Lubbock of a portion of the sampled wastewater.
h. Beginning with samples collected on June 28-30, 1982 fluorescent Pseudomonas sp. was substituted for Staphylococcus aureus as part of limited bacterial screen.
i. HeLa cells used for the assay were contaminated; results could not be obtained.
j. Membrane filtration technique.
k. Contaminated.
l. Fungal contamination at lower dilutions.
m. Enrichment procedure (for samples after November 1, 1982).
n. Assayed on Cetrimide agar (for samples after November 1, 1982).
o. Analysis not performed.
+ Presence of Salmonella (>1 colony/100 mL)
- Salmonella not detected (<1 colony/100 mL)

TABLE P-2. MICROORGANISM CONCENTRATIONS IN HANCOCK RESERVOIR

	Sampling date					
	1982					
	Jun 14-15	Jun 29-30	Jul 26-27	Aug 9-10	Aug 30-31	Sep 13-14
Source reservoir	1	1	1	1	1	1
Sample type[a]	C	C	C	C	C	C
Bacteria (cfu/mL)						
Standard plate count			36,000			
Total coliforms			500			
Fecal coliforms	520	60	190	390	10	350
Fecal streptococci	20	3	3	6.6	0.3	10
Mycobacteria sp.	4,000	200	<10		1,000	550
Clostridium perfringens[b]						
- vegetative			430			
- sporulated			4			
Staphylococcus aureus			<3			
Salmonella sp.		<0.01	<0.01	<0.01	<0.01	<0.01
Shigella sp.		<0.01	<0.01	<0.01	<0.01	<0.01
Yersinia enterocolitica		<0.01	<0.01	<0.01	<0.01	<0.01
Campylobacter jejuni		<10	<3	<3	<3	<10
Candida albicans		<10	<3	<3	<3	<10
Fluorescent Pseudomonas sp.		230[c]	13	16	2,000	250
Klebsiella sp.		10	30	130	<50	1,000
Viruses (pfu/mL)						
Bacteriophage	14	19	0.9		0.8	
Enteroviruses						
HeLa, 5 day (uncorrected)	0.002	0.014	<0.002	0.002	d	<0.002
HeLa, polio-neutralized	0.005	0.056	<0.002	0.004	d	0.002
RD, polio-neutralized	<0.002	<0.017	0.004	0.004	<0.002	0.008
Poliovirus concentration efficiency (%)	81	71	100	87	61	27
Physical Analyses (mg/L)						
Total organic carbon	33	21	14	27	23	28
Total suspended solids	218	67	21	24	24	44
Total volatile suspended solids	50	28	20	21	19	34
pH	7.6	7.9	8.0	8.1	7.9	8.4

continued...

TABLE P-2. (CONT'D)

	Sampling date						
	1982		1983				
	Nov 1-2	Dec 13-14	Feb 16-17	Mar 7-8	Mar 21-22	Apr 4-5	Apr 18-19
Source reservoir	1,3	1	1	1	1,2	1	1,2
Sample type[a]	G	G	C	C	G	C	G
Bacteria (cfu/mL)							
Standard plate count							
Total coliforms	1,000	10,000	500	100	2,100	2,100	20,000
Fecal coliforms	3.5	730	15	4	100	100	440
Fecal streptococci	0.1	23	14	2	19	29	30,000
Mycobacteria sp.							
Clostridium perfringens[e]							
- vegetative					0.60		<5.0[f]
- sporulated					<1.0		<5.0[f]
Staphylococcus aureus							
Salmonella sp.	-	-	-	-	-	-	-
Shigella sp.[g]					<0.01		<0.01
Yersinia sp.[g]					<0.01		<0.01
Campylobacter jejuni							
Candida albicans							
Fluorescent Pseudomonas sp.[h]					10		50
Klebsiella sp.					400		400
Viruses (pfu/mL)							
Bacteriophage					13		65
Enteroviruses							
HeLa, 5 day (uncorrected)	<0.004	0.020	0.002	<0.004	<0.004	<0.004	0.004
HeLa, polio-neutralized	<0.004	0.008	<0.004	[f]	<0.004	<0.004	<0.004
RD, polio-neutralized	<0.004	0.012	<0.004	0.004	0.004	<0.004	0.004
Poliovirus concentration efficiency (%)	1	i	76	83	50	45	94
Physical Analyses (mg/L)							
Total organic carbon	28	i	19	28	23	26	34
Total suspended solids	50	i	16	34	31	30	43
Total volatile suspended solids	42	i	12	25	18	17	32
pH	9.0	7.9	8.4	8.5	8.5	8.4	8.6

continued...

TABLE P-2. (CONT'D)

	Sampling date 1983					
	Jun 27-28	Jul 11-12	Jul 25-26	Aug 8-9	Aug 22-23	Sep 12-13
Source reservoir	1	1	1,2	1,3	1	1
Sample type[a]	C	C	G	G	G	C
Bacteria (cfu/mL)						
Standard plate count						
Total coliforms						
Fecal coliforms	300	150	3.0	110	30	15
Fecal streptococci	10	2.0	1.9	1.8	4.0	0.9
Mycobacteria sp.						
Clostridium perfringens[e]						
- vegetative						
- sporulated						
Staphylococcus aureus						
Salmonella sp.	+	-	-	-	-	-
Shigella sp.[g]						
Yersinia sp.[g]						
Campylobacter jejuni						
Candida albicans						
Fluorescent Pseudomonas sp.[h]						
Klebsiella sp.						
Viruses (pfu/mL)						
Bacteriophage						
Enteroviruses						
HeLa, 5 day (uncorrected)	0.004	<0.004	<0.004	<0.004	<0.004	<0.004
HeLa, polio-neutralized	<0.004	i	i	i	i	i
RD, polio-neutralized	<0.004	<0.004	0.008	<0.004	<0.004	<0.004
Poliovirus concentration efficiency (%)	57	41	42	25	24	70
Physical Analyses (mg/L)						
Total organic carbon	17	21	24	27	33	23
Total suspended solids	11	13	18	23	54	54
Total volatile suspended solids	6	8	17	17	46	33
pH	8.2	8.2	8.9	8.2	9.8	9.5

a G - Composite of grab samples from source reservoir; C - 24-hour composite of source reservoir.

b Most probable number (MPN)/mL.

c Beginning with samples collected on June 29-30, Fluorescent Pseudomonas sp. was substituted for Staphylococcus aureus as part of the limited bacterial screen.

d HeLa cells used for the assay were contaminated; results could not be obtained.

e Membrane filtration technique.

f Fungal contamination at lower dilutions.

g Enrichment procedure.

h Assayed on Cetrimide agar.

i Analysis not performed.

+ Presence of Salmonella (>1 colony/100 mL)

- Salmonella not detected (<1 colony/100 mL)

TABLE P-3. MICROORGANISM CONCENTRATIONS IN WILSON WASTEWATER

24-Hour composite samples analyzed	Sampling date 1980 Jun 3-4	 Jul 28-29	 1981 Jan 19-20	 Feb 16-17	 Mar 9-10	 Mar 23-24	 Apr 20-21
Bacteria (cfu/mL)							
Standard plate count	1,600,000	3,300,000					
Total coliforms	270,000	160,000	390,000	52,000	98,000	98,000	
Fecal coliforms	100,000	30,000	64,000	15,000	44,000	19,000	80,000
Fecal streptococci	6,800	2,300					
Mycobacteria sp.	1,400	1,900					
Clostridium perfringens[a]							
- vegetative	11,000	24,000					
- sporulated	1,500	240					
Staphylococcus aureus	33	<3.3					
Salmonella sp.	<0.004	<0.002					
Shigella sp.	<0.004	<0.002					
Yersinia enterocolitica	<0.002	<0.004					
Campylobacter jejuni							
Candida albicans							
Fluorescent Pseudomonas sp.	8,300	1,500					
Klebsiella sp.	100,000	70,000					
Viruses (pfu/mL)							
Bacteriophage	410	3,300	3,100				
Enteroviruses							
HeLa, 5 day (uncorrected)	0.047	15	<0.0009	0.22	0.002	0.001	0.003
HeLa, polio-neutralized							<0.001
RD, polio-neutralized							0.002
Poliovirus concentration efficiency (%)	56	47	55	69	46	42	76
Physical Analyses (mg/L)							
Total organic carbon	87	64	90	159	96	87	200
Total suspended solids	68	45	64	97	73	70	151
Total volatile suspended solids	39	29	54	77	58	51	89
pH	6.5	6.6	7.0	7.3	7.0	7.2	7.7

continued...

TABLE P-3. (CONT'D)

	Sampling date						
	1981						
24-Hour composite samples analyzed	May 4-5	May 18-19	Jun 1-2	Jun 15-16	Jun 29-30	Jul 20-21	Aug 17-18
Bacteria (cfu/mL)							
Standard plate count							
Total coliforms							
Fecal coliforms	41,000	66,000	110,000	110,000	36,000	54,000	53,000
Fecal streptococci							
Mycobacteria sp.							
Clostridium perfringens[a]							
- vegetative							
- sporulated							
Staphylococcus aureus						<10	<3
Salmonella sp.						≥0.01[d]	<0.1
Shigella sp.						<0.007	<0.008
Yersinia enterocolitica						<0.007	<0.008
Campylobacter jejuni						<10	<0.1
Candida albicans						<10	<3
Fluorescent Pseudomonas sp.							
Klebsiella sp.						56,000	20,000
Viruses (pfu/mL)							
Bacteriophage							
Enteroviruses							
HeLa, 5 day (uncorrected)	0.025	0.17	0.078	<0.001	0.99	0.006	0.013
HeLa, polio-neutralized	<0.001	0.004	0.0015	<0.014	0.008	0.002	0.001
RD, polio-neutralized	1.5	0.14	b	0.075	0.058	0.053	1.5
Poliovirus concentration efficiency (%)	32	c	74	55	42	53	63
Physical Analyses (mg/L)							
Total organic carbon	92	108	57	56	97	101	80
Total suspended solids	75	80	44	30	26	57	30
Total volatile suspended solids	60	59	36	26	22	42	23
pH	7.8	6.5	6.4	6.5	7.6	7.3	6.9

continued...

TABLE P-3. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1981 Sep 14-15	Nov 17-18	1982 Feb 15-16	Mar 1-2	Mar 8-9	Mar 22-23	Apr 5-6
Bacteria (cfu/mL)							
Standard plate count							
Total coliforms							
Fecal coliforms	8,700	44,000	17,000	130,000	140,000	81,000	110,000
Fecal streptococci							
Mycobacteria sp.							
Clostridium perfringens[a]							
- vegetative							
- sporulated							
Staphylococcus aureus	<3	<3	10,000		<3		<3
Salmonella sp.	≥0.006	≥1	<0.01		<0.01		<0.01
Shigella sp.	<0.006	<0.005	<0.01		<0.01		<0.01
Yersinia enterocolitica	<0.006	<0.005	<0.01		<0.01		<0.01
Campylobacter jejuni	<3	<3	<3		<3		<3
Candida albicans	<3	<3	<3		<3		<3
Fluorescent Pseudomonas sp.							
Klebsiella sp.	7,500	130,000	50,000		100,000		1,000
Viruses (pfu/mL)							
Bacteriophage							
Enteroviruses							
HeLa, 5 day (uncorrected)	0.001	0.06	<0.0007	<0.0008	0.12	0.11	1.5
HeLa, polio-neutralized	<0.001	<0.002	<0.001	e		<0.002	0.085
RD, polio-neutralized	1.0	0.15	<0.003		0.012		b
Poliovirus concentration efficiency (%)	50	96	233	87	74	86	77
Physical Analyses (mg/L)							
Total organic carbon		72	102	92	103	87	89
Total suspended solids	75	60	82	98	82	70	72
Total volatile suspended solids	57	50	73	74	76	67	59
pH	7.4	7.4	7.5	7.2	7.2	7.3	7.7

continued...

TABLE P-3. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1982 Apr 19-20	May 3-4	May 17-18	Jun 14-15	Jun 29-30	Jul 19-20
Bacteria (cfu/mL)						
Standard plate count						
Total coliforms						
Fecal coliforms	270,000	37,000	140,000	150,000	85,000	120,000
Fecal streptococci				8,200	6,500	
Mycobacteria sp.						
Clostridium perfringens[a]						
- vegetative						
- sporulated						
Staphylococcus aureus						
Salmonella sp.					≥0.01	≥0.01
Shigella sp.					<0.01	<0.01
Yersinia enterocolitica					<0.01	<3
Campylobacter jejuni					<10	<3
Candida albicans					<10	<1
Fluorescent Pseudomonas sp.					11,000[f]	9,300
Klebsiella sp.					16,000	35,000
Viruses (pfu/mL)						
Bacteriophage				1,300	1,500	
Enteroviruses						
HeLa, 5 day (uncorrected)	0.27	0.70	0.0076	<0.002	0.034	0.44
HeLa, polio-neutralized	0.003	0.008	<0.002	<0.002	0.036	0.004
RD, polio-neutralized	0.0045	0.008	<0.003	<0.002	0.036	0.004
Poliovirus concentration efficiency (%)	58	72	72	61	58	170
Physical Analyses (mg/L)						
Total organic carbon	92	68	81	75	69	76
Total suspended solids	74	89	60	67	70	44
Total volatile suspended solids	65	69	50	56	61	41
pH	7.6	7.5	7.5	7.2	7.0	7.5

continued...

TABLE P-3. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1982 Aug 9-10	Aug 30-31	Sep 13-14	Sep 27-28	Oct 11-12
Bacteria (cfu/mL)					
Standard plate count					
Total coliforms					
Fecal coliforms	130,000	120,000	81,000	18,000	51,000
Fecal streptococci					
Mycobacteria sp.					
Clostridium perfringens[a]					
- vegetative					
- sporulated					
Staphylococcus aureus					
Salmonella sp.	≥0.1	≥1	≥0.01	≥0.1	<0.01
Shigella sp.	<0.01	<0.01	<0.01	<0.1	<0.01
Yersinia enterocolitica	<0.01	<0.01	<0.01	<0.01	<0.01
Yersinia intermedia					≥1,000
Campylobacter jejuni	<3	<3	<10	<10	<10
Candida albicans	<3	<3	<300	<0.1	<10
Fluorescent Pseudomonas sp.	9,700	11,000	9,500	30,000	750
Klebsiella sp.	36,000	26,000	30,000	350,000	40,000
Viruses (pfu/mL)					
Bacteriophage					
Enteroviruses					
HeLa, 5 day (uncorrected)	0.058	g	0.61	0.043	0.008
HeLa, polio-neutralized	0.012	g	0.85	0.045	<0.002
RD, polio-neutralized	0.007	0.016	0.013	0.036	0.052
Poliovirus concentration efficiency (%)	117	47	33		92
Physical Analyses (mg/L)					
Total organic carbon	83	93	81	81	89
Total suspended solids	59	66	54	123	27
Total volatile suspended solids	49	55	48	70	25
pH	7.5	7.3	7.5	7.5	7.6

continued...

TABLE P-3. (CONT'D)

24-Hour composite samples analyzed	1982 Nov 1-2	1982 Dec 13-14^{h}	1983 Feb 16-17^{i}	1983 Mar 7-8	1983 Mar 21-22	1983 Apr 4-5	1983 Apr 18-19	1983 May 16-17
Bacteria (cfu/mL)								
Standard plate count								
Total coliforms	670,000	710,000	220,000	750,000	430,000	710,000	730,000	440,000
Fecal coliforms	130,000	110,000	14,000	150,000	76,000	150,000	130,000	350,000
Fecal streptococci	12,000	1,600	9,000	100,000	2,800	25,000	5,000	10,000
Mycobacteria sp.								
Clostridium perfringens								
- vegetative								
- sporulated								
Staphylococcus aureus								
Salmonella sp.	-	-	-	-	+	-	-	-
Shigella sp.								
Yersinia enterocolitica								
Campylobacter jejuni								
Candida albicans								
Fluorescent Pseudomonas sp.								
Klebsiella sp.								
Viruses (pfu/mL)								
Bacteriophage								
Enteroviruses								
HeLa, 5 day (uncorrected)	0.012	0.096	0.004^{b}	0.004	0.160	0.028	0.190	0.096
HeLa, polio-neutralized	<0.004	0.056	j	<0.004	0.049	0.040	0.031	0.004
RD, polio-neutralized	<0.004	0.020	j	<0.004	<0.004	<0.004	0.044	0.004
Poliovirus concentration efficiency (%)	j	j	12	86	59	89	24	89
Physical Analyses (mg/L)								
Total organic carbon	80	j	84	119	88	205	84	95
Total suspended solids	51	j	40	153	118	721	185	167
Total volatile suspended solids	48	j	33	130	99	504	148	132
pH	7.5	7.6	7.6	7.8	7.5	7.4	7.6	7.4

continued...

TABLE P-3. (CONT'D)

24-Hour composite samples analyzed	Sampling date 1983 Jun 27-28	Jul 11-12	Jul 25-26	Aug 8-9	Aug 22-23	Sep 12-13	Sep 27-28
Bacteria (cfu/mL)							
Standard plate count							
Total coliforms							
Fecal coliforms	260,000	370,000	240,000	310,000	230,000	530,000	260,000
Fecal streptococci	34,000	250	5,100	7,000	9,000	12,000	20,000
Mycobacteria sp.							
Clostridium perfringens							
- vegetative							
- sporulated							
Staphylococcus aureus							
Salmonella sp.	-	-	+	-	-	-	-
Shigella sp.							
Yersinia enterocolitica							
Campylobacter jejuni							
Candida albicans							
Fluorescent Pseudomonas sp.							
Klebsiella sp.							
Viruses (pfu/mL)							
Bacteriophage							
Enteroviruses							
HeLa, 5 day (uncorrected)	3.8	0.40	0.44	0.29	0.15	0.30	0.032
HeLa, polio-neutralized	5.0	0.52	0.15	0.15	0.26	0.028	0.11
RD, polio-neutralized	<0.004	0.008	<0.004	0.016	0.10	<0.004	<0.004
Poliovirus concentration efficiency (%)	62	42	44	36	78	65	43
Physical Analyses (mg/L)							
Total organic carbon	71	78	71	67	84	41	82
Total suspended solids	170	95	126	123	186	26	139
Total volatile suspended solids	126	75	100	94	130	22	101
pH	7.6	7.5	7.5	7.8	7.6	7.1	7.5

a Most probable number (MPN)/mL.

b Toxic concentrate.

c Sample lost--tube broken during handling.

d A new procedure was used for detection of Salmonella spp. (Kaper et al., Appl. Environ. Microbiol., 33:829-35, 1977) beginning in July 1981.

e Not done; no pfu were recovered on HeLa monolayers.

f Beginning with samples collected on June 29-30, Fluorescent Psuedomonas sp. was substituted for Staphylococcus aureus as part of the limited bacterial screen.

g HeLa cells used for the assay were contaminated; results could not be obtained.

h From this date on influent sampled instead of effluent.

i Sample arrived frozen.

j Analysis not performed.

+ Presence of Salmonella (>1 colony/100 mL)

- Salmonella not detected (<1 colony/100 mL)

TABLE P-4. BACTERIAL SCREENS[a]--WILSON, TEXAS

Organism	Sampling date Jun 3-4, 1980	 Jul 28-19, 1980
ENTEROBACTERIACEAE (10^3 cfu/mL)		
Citrobacter diversus	5	-
Citrobacter freundii	30	10
Citrobacter sp., other	5	-
Enterobacter agglomerans	20	30
Enterobacter cloacae	30	30
Enterobacter sakazakii	5	-
Escherichia coli	40	90
Hafnia alvei	5	-
Klebsiella oxytoca	55	-
Klebsiella ozaenae	5	10
Klebsiella pneumoniae	5	10
Serratia liquefaciens	10	-
Serratia rubidaea	5	-
Yersinia enterocolitica	5	-
NON-ENTEROBACTERIACEAE (10^3 cfu/mL)		
Achromobacter sp.	5	-
Achromobacter xylosoxidans	-	20
Aeromonas hydrophila	150	120
Alcaligenes sp.	5	20
CDC Group II K-2	5	-
Eikenella corrodens	20	-
Morgenella morgani	5	-
Pasteurella multocida	5	10
Pseudomonas cepacia	15	-
Pseudomonas fluorescens	15	-
Pseudomonas putida	15	50
Pseudomonas putrefaciens	25	-
Pseudomonas sp., other	45	500

[a] Highest levels observed on either MacConkey agar or brilliant green agar and identified by API 20E biochemical tests.

TABLE P-5. VIRUSES ISOLATED FROM LUBBOCK EFFLUENT DURING BASELINE YEARS[a]

Assay	Jun 3-4 (1980)	Jul 28-29 (1980)	Nov 3-4 (1980)	Apr 20-21 (1981)	Jun 15-16 (1981)	Jul 20-21 (1981)	Aug 17-18 (1981)
HeLa (unaltered concentrate)							
Concentration (pfu/L)	780	1,200	730	57	100[b]	65	45
Virus type							
Polio 1	2			1	1		3
Polio 2	1			16	1	6	4
Polio 3	1			7	3		4
Coxsackie A1	1						
Coxsackie A7	1						
Coxsackie A16	1						
Coxsackie B1					4		
Coxsackie B3	20	14	16		1		1
Coxsackie B4	3						
Coxsackie B5	19	2	4		25	11	
Echo 1	1						
Echo 3	1						
Echo 6	1						
Echo 11	4			1	1		
Echo 14					1		
Echo 21	2						
Echo 24	1						
Echo 25	1						
Echo 30					1		
Unidentified	21	2			4	4	1
TOTAL SAMPLED	81	18	20	25	42	21	13
HeLa (polio-neutralized)							
Concentration (pfu/L)			300	18	65[b]	20	5.3
Virus Type							
Polio 2							1
Coxsackie B3			19				1
Coxsackie B5				4		11	1
Echo 14							1
Unidentified				2			
TOTAL SAMPLED			19	6		11	4
RD (polio-neutralized)							
Concentration (pfu/L)				8	150	93	420
Virus type							
Coxsackie A16					1		
Coxsackie B4					1		
Echo 5					3	1	5
Echo 7						6	2
Echo 11				2	1	4	1
Echo 12							1
Echo 13							1
Echo 15						1	
Echo 19						3	
Echo 20							1
Echo 24							1
Echo 27						1	
Echo 31							1
Unidentified				2	5	2	3
TOTAL SAMPLED				4	11	18	16

[a] Plaque forming units on cell monolayers.
[b] Labeling error precluded separating neutralized/unaltered viruses.

TABLE P-6. VIRUSES ISOLATED FROM WILSON EFFLUENT DURING BASELINE YEARS[a]

	Sampling date			
	1980		1981	
Assay	Jun 3-4	Jul 28-29	Jun 15-16	Aug 17-18
HeLa (unaltered concentrate)				
Concentration (pfu/L)	47	15,000	<1	13
Virus type				
Polio 1	2			
Polio 2				9
Polio 3	16			
Coxsackie A10	1			
Coxsackie B3		12		
Echo 2				1
Echo 25	1			
Unidentified	5	3	1	2
TOTAL SAMPLED	25	15	0	12
HeLa (polio-neutralized)				
Concentration (pfu/L)			<1.4	1.0
Virus type				
RD (polio-neutralized)				
Concentration (pfu/L)			75	1500
Virus type				
Polio 2			1	
Coxsackie A9				1
Echo 5			5	
Echo 31			1	
Unidentified			1	6
TOTAL SAMPLED			9	7

[a] Plaque forming units on cell monolayers.

TABLE P-7. VIRUSES ISOLATED FROM WILSON EFFLUENT DURING 1982

	Sampling date				
Assay	Mar 8-9	Apr 5-6	Jun 29-30	Aug 9-10	Sep 13-14
HeLa (unaltered concentrate)					
Concentration (pfu/L)	120	1500	34	58	610
Virus type					
Polio 1	1	1	1	8	1
Polio 2	10	23		3	
Polio 3	8		1	6	
Coxsackie B5			10	3	20
Echo 11				1	
Echo 24				2	
Unidentified	—	1	—	1	4
TOTAL SAMPLED	19	25	12	24	25
HeLa (polio-neutralized)					
Concentration (pfu/L)	<2	85	36	12	850
Virus type					
Polio 2		4			
Coxsackie B4			2		
Coxsackie B5			10	2	14
Echo 11				1	
Unidentified		1	1	—	—
TOTAL SAMPLED		5	13	3	14
RD (polio-neutralized)					
Concentration (pfu/L)	12	a	36	6.6	13
Virus type					
Echo 13			3		
Unidentified			3	1	
TOTAL SAMPLED			6	1	

a Toxic sample.

TABLE P-8. VIRUSES ISOLATED FROM WILSON INFLUENT DURING 1983

Assay	Sampling Date 1983 Feb 16-17	Mar 21-22	Apr 18-19	May 16-17	Jul 11-12	Aug 8-9	Sep 12-13	Sep 26-27
HeLa (unaltered concentrate)								
Concentration (pfu/L)	4	160	190	96	400	290	300	32
Virus type								
Polio 1		5		9		6	24	
Polio 2			5	1				
Polio 3			6	6			1	
Coxsackie A13		8	1					
Coxsackie B2		1	2				1	8
Coxsackie B5		1	3	1	27	5		
Echo 7						1		
Echo 25		1						
Echo 26						1		
Echo 27						7		
Echo 29						1		
Unidentified	1	3	1	3		1		
TOTAL SAMPLED	1	19	18	20	27	22	26	8
HeLa (neutralized)								
Concentration (pfu/L)	<6	49	31	4	520	150	28	110
Virus Type								
Coxsackie B2			2				6	12
Coxsackie B3		2						
Coxsackie B5			4	1		16		
Unidentified		9	1				1	
TOTAL SAMPLED		11	7	1		16	7	12

TABLE P-8. GEOMETRIC MEAN OF MICROORGANISM CONCENTRATIONS IN WILSON WASTEWATER

Sampling period Number of samples	May/Jun 80 6-3 1	Summer 80 7-28 1	Fall-Win 80 1-19 1	Spring 81 2-16/4-20 4	May/Jun 81 5-4/6-15 4	Summer 81 6-29/9-14 4	Fall-Win 81 11-17/2-15 2
Bacteria (cfu/mL)							
Standard plate count	1,600,000	3,300,000					
Total coliforms	270,000	160,000	390,000	79,000			
Fecal coliforms	100,000	30,000	64,000	32,000	76,000	31,000	27,000
Fecal streptococci	6,800	2,300					
Viruses (pfu/mL)							
Bacteriophage	410	3,300	3,100				
Enteroviruses							
HeLa, 5-day (uncorrected)	0.047	15	<0.0009	0.006	0.068	0.017	0.03
HeLa, polio-neutralized				<0.001	0.001	0.003	<0.002
RD, polio-neutralized				0.002	0.25	0.26	0.08
Physical Analyses (mg/L)							
Total organic carbon	87	64	90	128	75	92	86
Total suspended solids	68	45	64	83	53	43	70
Total volatile suspended solids	39	29	54	67	43	33	60
pH	6.5	6.6	7.0	7.3	6.8	7.3	7.4

continued...

TABLE P-9. (CONT'D)

	Spring 82	May/Jun 82	Summer 82	Fall-Win 82	Spring 83	May/Jun 83	Summer 83
Sampling period	3-1/4-19	5-3/7-19	8-9/9-13	9-27/12-13	2-16/4-18	5-16/6-27	7-11/9-27
Number of samples	5	5	3	4	5	2	6
Bacteria (cfu/mL)							
Standard plate count							
Total coliforms				690,000	520,000	440,000	
Fecal coliforms	130,000	95,000	110,000	60,000	79,000	300,000	310,000
Fecal streptococci		7,300^a		4,400^a	13,000	18,000	5,200
Viruses (pfu/mL)							
Bacteriophage		1,400^a					
Enteroviruses							
HeLa, 5-day (uncorrected)	0.40	0.094	0.188	0.025	0.027	0.60	0.20
HeLa, polio-neutralized	0.016	0.010	0.10	0.025	0.030	0.14	0.15
RD, polio-neutralized	0.007	0.005	0.011	0.010	0.011	<0.004	0.021
Physical Analyses (mg/L)							
Total organic carbon	92	74	86	83	108	82	69
Total suspended solids	79	64	60	55	157	168	100
Total volatile suspended solids	68	55	51	44	126	129	77
pH	7.4	7.3	7.4	7.5	7.6	7.5	7.5

a Based on two samples.

TABLE P-10. WASTEWATER SAMPLES COLLECTED DURING 1982 AEROSOL MONITORING (30 MINUTE COMPOSITES)
WASTEWATER FROM PIPELINE DURING SPRING IRRIGATION PERIOD

Parameter	Sampling date/aerosol run							
	Feb 22 M1	Feb 23 M2	Feb 24 M3	Mar 15 Q1	Mar 16 V1	Mar 17 M4	Mar 18 M5	Mar 19 M6
Bacteria (cfu/mL)								
Fecal coliforms	100,000	1,000,000	110,000	51,000	81,000	39,000	57,000	68,000
Fecal streptococci	4,400	7,200	6,300	4,800	4,500	1,900	5,800	16,000
Clostridium perfringens[a]								
Vegetative		360						
Sporulated		360						
Mycobacteria sp.	16,000	18,000	45,000	13,000	20,000	29,000	13,000	15,000
Viruses (pfu/mL)								
Bacteriophage	1,200	1,500	1,400	1,100	840	530	1,100	940
Enteroviruses (uncorrected)								
HeLa, 5 day	0.054	0.093	0.047	0.067	0.16	0.11	0.12	0.028
HeLa, polio-neutralized	0.015	0.024		0.0084	0.035	0.022	0.047	0.0023
RD, polio-neutralized	0.051	0.012		0.034	0.030	0.067		
Poliovirus concentration efficiency (%)	57	49	76	60		64	49	63
Physical Analyses (mg/L)								
Total organic carbon	135	161	168	92	100	158	128	164
Total suspended solids	147	182	217	87	101	245	92	185
Total volatile suspended solids	121	152	176	74	90	207	90	160
Sample conditions								
pH	6.9	6.8	7.0	7.2	7.4	7.0	7.1	7.1
Temperature (°C)	2	3	8	9	9	4	2	5

a Membrane filtration procedure used to enumerate C. perfringens in aerosol-related samples.

TABLE P-11. WASTEWATER SAMPLES COLLECTED DURING 1982 AEROSOL MONITORING (30 MINUTE COMPOSITES) WASTEWATER FROM PIPELINE DURING SUMMER IRRIGATION PERIOD

	Sampling date/aerosol run						
	Jul 7	Jul 8	Jul 13	Jul 14	Jul 15	Aug 2	Aug 3
Parameter	M7[a]	M8	Q2	M11	M12	V2	M14
Bacteria (cfu/mL)							
Fecal coliforms	44,000	31,000	50,000	13,000	76,000	180,000	37,000
Fecal streptococci	4,200	3,200	3,600	4,600	5,600	2,000	4,900
Clostridium perfringens[b]							
Vegetative							
Sporulated							
Mycobacteria sp.	100,000	550,000	25,000	11,000	10,000	4,000	5,300
Viruses (pfu/mL)							
Bacteriophage	1,700	930	720	16	1,100	880	1,900
Enteroviruses (uncorrected)							
HeLa, 5 day	0.54	0.51	0.14	0.013	0.078	0.10	1.5
HeLa, polio-neutralized	0.47	0.55	0.067	0.002	0.097	0.10	0.10
RD, polio-neutralized	0.17	0.26	0.020	0.016	0.018	0.004	0.011
Poliovirus concentration efficiency (%)	64	57	39	50	49	80	214
Physical Analyses (mg/L)							
Total organic carbon	128	92	76	51	80	52	71
Total suspended solids	307	213	82	67	170	79	86
Total volatile suspended solids	213	161	66	54	119	62	68
Sample conditions							
pH	7.0	7.3	7.4	7.6	7.6	7.4	7.5
Temperature (°C)	1	10	3	3	5	2	2

continued...

TABLE P-11. (CONT'D)

Parameter	Aug 4 V3	Aug 5 M15	Aug 23 M17[c]	Aug 24 V4[c]	Aug 25 M18	Aug 27 M20[c]
	Sampling date/aerosol run					
Bacteria (cfu/mL)						
Fecal coliforms	5,600	30,000	16,000	93	29,000	360
Fecal streptococci	2,600	2,700	300		830	10
Clostridium perfringens[b]						
Vegetative			460	<5.0	360	93
Sporulated			200	5.0	190	230
Mycobacteria sp.	2,300	6,000				
Viruses (pfu/mL)						
Bacteriophage	1,600	1,200	820	150	2,100	140
Enteroviruses (uncorrected)						
HeLa, 5 day	2.2	0.21	0.39	0.066	0.10	0.044
HeLa, polio-neutralized	0.060	0.080	0.34	0.051	0.11	0.13
RD, polio-neutralized	0.020	0.022	0.34	0.008	0.28	0.28
Poliovirus concentration efficiency (%)	350	94		85		
Physical Analyses (mg/L)						
Total organic carbon	66	65	58	61	46	63
Total suspended solids	93	69	62	58	48	49
Total volatile suspended solids	75	57	49	47	36	41
Sample conditions						
pH	7.5	7.8	7.2	7.3	7.4	7.3
Temperature (°C)	3	2	2	3	3	3

a Presumed pipeline source based on microbial parameters.

b Membrane filtration procedure used to enumerate C. perfringens in aerosol-related samples.

c Chlorinated.

TABLE P-12. WASTEWATER SAMPLES COLLECTED DURING 1982 AEROSOL MONITORING (30 MINUTE COMPOSITE) WASTEWATER FROM RESERVOIR DURING SUMMER CROP IRRIGATION

	Sampling date/aerosol run				
Parameter	Jul 9 M9	Jul 11 M10	Jul 16 M13	Aug 6 M16	Aug 26 M19
Bacteria (cfu/mL)					
Fecal coliforms	230	40	1,100	450	750
Fecal streptococci	30	13	53	3.0	3.0
Clostridium perfringens[a]					
Vegetative					3.0
Sporulated					<1.0
Mycobacteria sp.	430	100	230	10	
Viruses (pfu/mL)					
Bacteriophage	1.2	0.40	15	2.4	5.3
Enteroviruses (uncorrected)					
HeLa, 5 day	0.034	0.002	0.004	0.12	8.7
HeLa, polio-neutralized	0.002	<0.002	0.013	0.008	0.006
RD, polio-neutralized	<0.002	<0.002	0.002	0.002	<0.002
Poliovirus concentration efficiency (%)	61	71	52	108	
Physical Analyses (mg/L)					
Total organic carbon	19	16	16	43	17
Total suspended solids	26	27	21	35	12
Total volatile suspended solids	26	24	19	35	12
Sample conditions					
pH	8.2	8.0	7.8	8.5	7.9
Temperature (°C)	5	1	8	2	2

[a] Membrane filtration procedure used to enumerate C. perfringens on aerosol-related samples.

TABLE P-13. SOURCE STRENGTH OF RHODAMINE IN WASTEWATER DURING DYE RUNS

Dye Run	Rhodamine concentration in wastewater sample, mg/L								
	Min 0	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6	Min 7	Min 8
D1	96	126	183	95	10	12	25		
D2	53	94	93	91	91	91	87	88	9.0
D3	112	119	118	108	110	102	99		
D4	95	111	109	112	115	113	105	6.7	

TABLE P-14. RHODAMINE AEROSOL CONCENTRATION DURING DYE RUNS

Dye run	Tower	Rhodamine concentration in air, 10^{-6} μg/m^3					
		Near pairs			Far pairs		
		(Dist)	L	R	(Dist)	L	R
D1	3	(31 m)	22	4.5	(81 m)	0.38	1.5
	5	(40 m)	1.1	0.89	(115 m)	1.1	0.96
D2	6	(25 m)	80	0.46	(75 m)	0.67	0.87
	4	(25 m)	1.9	7.5	(75 m)	2.3	1.3
D3	6	(25 m)	2.3	9.7	(75 m)	0.71	0.50
	4	(25 m)	3.7	0.47	(75 m)	1.9	0.79
D4	5	(40 m)	3.7	6.3	(80 m)	1.3	2.4
	3	(40 m)	2.5	2.4	(80 m)	1.0	1.8

TABLE P-15. SAMPLED STANDARD PLATE COUNT IN AIR BY PARTICLE SIZE

Run no. Run date Run time	Andersen sampler stage	Range of particle sizes (μ)	Standard plate count concentration in air by particle size, cfu/m^3							
			Upwind		Downwind of irrigation nozzle line					
			L	R	L	R	L	R	L	R
					36 m		61 m		75 m	
P1	1	≥7.0	81	170	260	240	200	280	290	140
2-23-82	2	4.7-7.0	47	65	130	190	140	150	110	150
1609-1619	3	3.3-4.7	68	86	240	540	280	70	240	180
	4	2.1-3.3	72	65	300	140	TNTC[a]	280	170	170
	5	1.1-2.1	60	60	180	140	220	250	TNTC	170
	6	0.65-1.1	TNTC	TNTC	82	100	70	90	74	TNTC
					33 m		58 m		83 m	
P2[b]	1	≥7.0	260	94	1700	2300	210	1700	290	110
3-16-82	2	4.7-7.0	60	64	1200	2500	210	540	920	140
1539-1549	3	3.3-4.7	22	64	1300	1500	960	1200	450	180
	4	2.1-3.3	78	47	390	650	130	920	190	120
	5	1.1-2.1	95	210	190	100	43	180	120	TNTC
	6	0.65-1.1	22	43	29	20	TNTC	78	TNTC	TNTC
					20 m		45 m		70 m	
P3	1	≥7.0	16	370	1080	TNTC	TNTC	1500	350	528
7-8-82	2	4.7-7.0	27	330	1200	1300	380	590	180	169
1510-1518	3	3.3-4.7	38	400	340	650	240	200	59	77
	4	2.1-3.3	38	180	120	87	68	87	74	67
	5	1.1-2.1	11	290	95	130	15	110	53	56
	6	0.65-1.1	32	37	5	<1	10	<1	48	10
					35 m		60 m		85 m	
P4	1	≥7.0	110	–[c]	1200	520	410	390	280	640
7-14-82	2	4.7-7.0	5	<1	660	550	390	690	64	180
1519-1527	3	3.3-4.7	5	37	TNTC	550	300	370	160	360
	4	2.1-3.3	<1	>540	290	150	83	150	43	72
	5	1.1-2.1	5	37	46	45	15	31	37	82
	6	0.65-1.1	16	11	10	110	29	CS	64	5

continued...

TABLE P-15. (CONT'D)

Run no. Run date Run time	Andersen sampler stage	Range of particle sizes (μ)	Standard plate count concentration in air by particle size, cfu/m³							
			Upwind		Downwind of irrigation nozzle line					
			L	R	L	R	L	R	L	R
					35 m		60 m		85 m	
P5	1	≥7.0	1000	410	TNTC	TNTC	600	880	640	490
8-25-82	2	4.7-7.0	160	540	TNTC	TNTC	400	1000	520	630
1730-1738	3	3.3-4.7	TNTC	11	1420	TNTC	640	250	370	270
	4	2.1-3.3	150	150	880	490	190	160	140	250
	5	1.1-2.1	100	11	310	150	130	87	140	110
	6	0.65-1.1	59	160	26	5	180	26	43	31

CS - fungal contamination

a TNTC - either too numerous to count (>2500 cfu/m^3 for P1 to P3; >1500 cfu/m^3 for P4 and P5) or fungal contamination. For data summary, it was assumed that TNTC = 3000 cfu/m^3 for P1 to P3 and TNTC = 2000 for P4 and P5 when values from paired sampler and/or adjoining stages were large. When these neighboring values were low, presumed fungal contamination TNTC was assumed to equal the value of the same stage for the paired sampler or the average of the adjoining stages.

b Standard plate count of wastewater = 5.1 x 10^8 cfu/mL

c Sample lost.

TABLE P-16. MICROORGANISM DENSITIES IN AIR ON BACKGROUND AIR RUNS[a]

Back-ground run no.	Sampler location[b]								
	Wilson A	Wilson B	Wilson C	Effluent pond D	Rural E	Rural F	Rural G	Rural H	Rural I
Standard Plate Count (cfu/m^3)									
B1	-	1150	260	1900	2800	CS	390	190	CS
B2	530	680	CS	430	1220	990	CS	3500	450
B3	1050	CS	500	370	280	1030	-	200	260
B4	CS	430	630	73	65	130	60	CS	500
Fecal Coliforms (cfu/m^3)									
B1	-	<0.4	<0.1	<0.2	<0.3	<0.1	<0.1	<0.2	<0.4
B2	<0.1	<0.1	<0.4	<0.1	<0.2	<0.3	<0.2	<0.2	<0.1
B3	<0.1	<0.2	<0.1	<0.3	<0.1	0.3	-	<0.1	<0.4
B4	<0.1	<0.1	<0.2	<0.1	<0.1	<0.1	<0.1	<0.2	<0.1
Fecal Streptococci (cfu/m^3)									
B1	-	0.5	8.0	<0.1	<0.2	1.1	0.1	<0.1	0.3
B2	0.9	0.3	2.1	0.3	0.2	1.3	0.3	0.3	0.1
B3	0.7	<0.1	0.3	0.2	0.2	2.3	-	<0.1	2.4
B4	11	0.6	0.3	0.3	0.3	1.5	0.2	0.8	0.2
Mycobacteria (cfu/m^3)									
B1	-	<0.2	0.1	<0.1	<0.2	<0.1	0.1	0.1	0.3
B2	<0.1	0.1	<0.3	<0.1	0.1	<0.2	<0.1	<0.1	<0.1
B3	<0.1	<0.1	0.1	<0.2	<0.1	<0.1	-	<0.1	0.5
B4	<0.1	<0.1	<0.1	3.4[c]	<0.1	<0.1	0.1	<0.1	<0.1
Coliphage (pfu/m^3)									
B1	-	<0.4	<0.1	<0.2	<0.3	<0.2	<0.1	<0.2	<0.4
B2	<0.2	<0.1	<0.4	<0.1	<0.2	<0.4	<0.2	<0.2	<0.1
B3	<0.1	<0.2	<0.1	<0.3	<0.1	<0.1	-	<0.1	<0.4
B4	<0.1	<0.1	<0.2	<0.2	<0.1	<0.1	<0.1	<0.2	<0.1

- - No sample collected.
CS - Contaminated sampler (presumed).

[a] Conducted August 5-8, 1980.

[b] Sampler locations shown in Figure 8.

[c] Cows grazing approximately 300 to 500 m upwind from sampling site.

TABLE P-17. SAMPLED FECAL COLIFORM DENSITIES ON THE MICROORGANISM AEROSOL RUNS

Aerosol run number	Fecal coliform concentration in wastewater (cfu/mL)	Fecal coliform concentration in air (cfu/m^3 of air)							
		Upwind of irrigation rig	Downwind of irrigation nozzle line						
			20-39 m	40-59 m	60-89 m	90-149 m	150-249 m	250-349 m	350-409 m
WASTEWATER FROM PIPELINE—SPRING 1982 IRRIGATION									
M1	100,000	<0.2 <0.2	>250		>250	21 15	3.2		
M2	1,000,000	<0.1 CS	150		110	2.3 2.1	<0.1 <0.1		
M3	110,000	<0.2 <0.4		190	330	36 26	40 13		
M4	39,000	<0.1 <0.1		0.2[a]	133[a]	<0.3 <0.1	<0.1 <0.1		
M5	57,000	<0.1 <0.1			120	CS	16 15	3.5 3.8	
M6	68,000	<0.2 <0.1		120	49	7.7 4.3	CS 0.1		
WASTEWATER FROM PIPELINE—SUMMER 1982 IRRIGATION									
M7[b]	44,000	<0.3 <0.7		140	83	14 10	3.7 3.2		
M8	31,000	<0.2 <0.3		900	137	0.3 1.2	<0.3 <0.3		
M11	13,000	<0.3 <0.3				CS	<0.3	<0.4 <0.6	<0.2 <0.3
M12	76,000	<0.3 CS				37	70	0.1 <0.3	0.6 0.2
M14	37,000	CS <0.1				<0.4	0.2	<0.1 <0.1	<0.2 <0.2
M15	30,000	<0.1 <0.1				<0.2	<0.2	<0.1 CS	<0.1 <0.3
M17[c,d]	16,000	<0.2 <0.2				0.5	2.7	3.5 CS	1.6 4.8
M18[d]	29,000	<0.2 <0.2				0.4	0.3	27[e]	0.6 4.8
M20[c]	360	CS <0.2				<0.1	<0.1	<0.1 <0.3	<0.1 <0.1
WASTEWATER FROM RESERVOIR—SUMMER 1982 IRRIGATION									
M9	230	<0.3 <0.3		<3.3	CS	<0.4 <0.3	<0.3 <0.3		
M10	40	<0.3 <0.3		CS	1.2	0.1 0.3	0.1 0.3	<0.3 <0.3	<0.4 <0.3
M13	1,100	CS <0.4[f]				CS	1.5		
M16	450	<0.1 CS		1.2	0.2	CS 0.3	2.2 2.0		
M19	750	<0.3 <0.3	15 CS	5.7 0.3		0.5 <0.2			

CS - contaminated sample.
<X - none detected at detection limit X

a Questionable result, excluded from summary tables.
b Presumed pipeline source, based on microbial parameters.
c Wastewater chlorinated at Lubbock treatment plant.
d Run conducted at night.
e Possible contamination.
f Fungal contamination.

TABLE P-18. SAMPLED FECAL STREPTOCOCCUS DENSITIES ON THE MICROORGANISM AEROSOL RUNS

Aerosol run number	Fecal streptococcus concentration in wastewater (cfu/mL)	Fecal streptococcus concentration in air (cfu/m^3 of air)							
		Upwind of irrigation rig	Downwind of irrigation nozzle line						
			20-39 m	40-59 m	60-89 m	90-149 m	150-249 m	250-349 m	350-409 m
WASTEWATER FROM PIPELINE—SPRING 1982 IRRIGATION									
M1	4,400	0.1 0.1	70[a]		60[a]	54 60[a]	42		
M2	7,200	0.1 CS	65		65	20 22	14 11		
M3	6,300	<0.1 0.3		69	110	38 33	45 47		
M4	1,900	<0.1 <0.1		70	600	12 6.3	4.3 3.7		
M5	5,800	<0.1 <0.1			95	230	100 110	23 20	
M6	16,000	<0.2 0.2		620	260	77 48	20 23		
WASTEWATER FROM PIPELINE—SUMMER 1982 IRRIGATION									
M7[b]	4,200	0.1 <0.7		140	120	63 20	19 18		
M8	3,200	0.1[c] 2.7[c]		670	130	7.4 8.9	3.7 1.8		
M11	4,600	<0.3 <0.3				CS	4.3	0.4 0.6	<0.2 0.1
M12	5,600	<0.3 CS				53	31	3.0 2.7	4.5 4.2
M14	4,900	<0.1 <0.1				1.2	0.2	0.1 3.3	<0.3 <0.2
M15	2,700	1.3 1.0				2.8	CS	0.3 1.0	1.2 0.9
M17[d,e]	300	<0.2 <0.2				2.4	0.6	0.1 0.4	<0.1 <0.1
M18[e]	830	0.2 <0.2				3.3	4.9	2.3	3.8 0.3
M20[d]	10	CS <0.2				0.1	<0.1	<0.1 <0.3	<0.1 0.1
WASTEWATER FROM RESERVOIR—SUMMER 1982 IRRIGATION									
M9	30	<0.3 <0.3		<3.3	<3.3	<0.4 <0.3	<0.3 <0.3		
M10	13	<0.3 <0.3		1.2	0.3	0.1 <0.3	<0.3 0.3		
M13	53	CS <0.4				2.2	3.2	<0.3 0.6	0.4 <0.3
M16	3	9.6[f] 0.1		0.5	<0.2	CS 0.1	<0.1 <0.1		
M19	3	<0.3 <0.3	0.3 CS	0.7 <0.3		<0.3 <0.3			

CS - contaminated sample.
<X - none detected at detection limit X.

a Assayed above usual counting range (>200 cfu/47 mm filter).
b Presumed pipeline source, based on microbial parameters.
c Possible contamination.
d Wastewater chlorinated at Lubbock treatment plant.
e Run conducted at night.
f Probable contamination, excluded from summary tables.

TABLE P-19. SAMPLED MYCOBACTERIA DENSITIES ON THE MICROORGANISM AEROSOL RUNS

Aerosol run number	Mycobacteria concentration in wastewater (cfu/mL)	Mycobacteria concentration in air (cfu/m^3 of air)							
		Upwind of irrigation rig	Downwind of irrigation nozzle line						
			20-39 m	40-59 m	60-89 m	90-149 m	150-249 m	250-349 m	350-409 m
WASTEWATER FROM PIPELINE—SPRING 1982 IRRIGATION									
M1	16,000	1.3 1.3	7.0		11	2.5 6.5	5.6		
M3	45,000	<0.1 <0.3		3.6	<0.4	1.7 3.0	6.7 <0.1		
M4	29,000	<0.1 <0.1		7.0	7.9	2.0 <0.1	<0.1 <0.1		
M5	13,000	CS CS			9.0	CS	4.7 1.5	4.1 CS	
M6	15,000	<0.1 <0.1		20	32	2.0 3.7	<0.2 <0.1		
WASTEWATER FROM PIPELINE—SUMMER 1982 IRRIGATION									
M7[a]	100,000	<0.1 <0.3		0.3	0.2	0.3 0.3	<0.3 0.2		
M8	550,000	<0.1 <0.2		1.4	<0.2	0.2 <0.1	0.2 <0.2		
M11	11,000	<0.1 <0.1				0.2	<0.2	<0.2 <0.3	<0.1 <0.1
M12	10,000	<0.2 <0.2				0.5	<0.2	0.4 0.6	CS <0.2
M14	5,300	<0.1 0.7				0.7	<0.2	<0.1 0.1	1.0 <0.2
M15	6,000	CS <0.1				5.0	<0.2	<0.1 0.4	<0.1 <0.3
WASTEWATER FROM RESERVOIR—SUMMER 1982 IRRIGATION									
M9	430	<0.1 <0.2		<0.2	<0.2	<0.2 0.1	<0.1 <0.1		
M10	100	<0.1 <0.1		CS	CS	<0.1 <0.1	<0.1 0.3		
M13	230	<0.2 <0.2				<0.2	CS	<0.1 <0.1	<0.2 <0.1
M16	10	<0.1 CS		0.2	<0.2	CS 0.9	<0.1 <0.1		

CS - contaminated sample.
<X - none detected at detection limit X

a Presumed pipeline source, based on microbial parameters.

TABLE P-20. SAMPLED CLOSTRIDIUM PERFRINGENS DENSITIES ON THE MICROORGANISM AEROSOL RUNS

Aerosol run number	Clostridium perfringens concentration in wastewater (cfu/mL)	Clostridium perfringens concentration in air (cfu/m^3 of air)							
		Upwind of irrigation rig	Downwind of irrigation nozzle line						
			20-39 m	40-59 m	60-89 m	90-149 m	150-249 m	250-349 m	350-409 m
M2-Pipeline									
Vegetative	360	<0.1 <0.2	8.2		9.3	2.8 1.7	1.6 1.7		
M17-Pipeline[a,b]									
Vegetative	460	<0.3 <0.3				9.8	6.7	3.9 1.4	0.5 2.6
Sporulated	200	<0.3 <0.3				1.8	0.9	1.6 0.8	1.4 <0.3
M18-Pipeline[b]									
Vegetative	360	<0.3 <0.3				1.3	4.1	2.7	2.3 3.2
Sporulated	190	<0.3 <0.3				<0.3	5.4	<0.3	0.5 0.5
M19-Reservoir									
Vegetative	3	<0.3 <0.3	<0.2 <0.3	<0.3 <0.3		<0.3 <0.3			
Sporulated	<1.0	<0.3 <0.3	<0.2 <0.2	<0.3 <0.3		<0.3 <0.3			
M20-Pipeline[a]									
Vegetative	93	<0.2 0.5				<0.1	<0.1	<0.1 0.3	<0.1 <0.1
Sporulated	230	<0.2 <0.2				0.2	<0.1	<0.1 <0.3	<0.1 <0.1

<X - none detected at detection limit X

a Wastewater chlorinated at Lubbock treatment plant.

b Run conducted at night.

TABLE P-21. SAMPLED COLIPHAGE DENSITIES ON THE MICROORGANISM AEROSOL RUNS

Aerosol run number	Coliphage concentration in wastewater (pfu/mL)	Coliphage concentration in air (pfu/m³ of air): Upwind of irrigation rig	Downwind of irrigation nozzle line: 20-39 m	40-59 m	60-89 m	90-149 m	150-249 m	250-349 m	350-409 m
WASTEWATER FROM PIPELINE—SPRING 1982 IRRIGATION									
M1	1,200	<0.1 <0.1	38		50	26 13	7.5		
M2	1,500	<0.1 <0.3	4.0		8.2	3.2 3.8	0.7 0.4		
M3	1,400	<0.1 <0.3		5.7	11	7.7 2.9	12 5.0		
M4	530	<0.1 <0.1		8.2	7.9	0.8 0.5	0.2 0.1		
M5	1,100	<0.1 <0.1			6.0	8.6	4.3 4.7	0.6 1.4	
M6	940	<0.1 <0.1		23	12	5.3 3.7	1.8 1.8		
WASTEWATER FROM PIPELINE—SUMMER 1982 IRRIGATION									
M7[a]	1,700	<0.1 <0.3		10	5.0	3.1 3.7	1.2 1.2		
M8	930	3.5[b] 5.7[b]		8.3	5.3	3.9 4.5	4.3 2.3		
M11	16	CS CS				CS	CS	CS CS	CS CS
M12	1,100	<0.1 0.1[c]				3.2	1.1	0.1 0.1	0.1 0.1
M14	1,900	<0.1 <0.1				<0.3	<0.1	<0.1 <0.1	<0.2 <0.1
M15	1,200	<0.1 <0.1				<0.1	<0.1	<0.1 <0.1	<0.1 <0.2
M17[d,e]	820	<0.5 <0.1				0.1	0.3	0.1 <0.1	<0.1 0.1
M18[e]	2,100	<0.1 <0.1				1.5	1.1	1.1	0.2 0.4
M20[d]	140	<0.1 <0.1				<0.1	<0.1	<0.1 <0.2	<0.1 <0.1
WASTEWATER FROM RESERVOIR—SUMMER 1982 IRRIGATION									
M9	1.2	<0.1 <0.1		<0.1	<0.1	<0.2 <0.1	<0.1 <0.1		
M10	0.4	<0.1 <0.1		<0.1	<0.1	<0.1 <0.1	<0.1 <0.2		
M13	15	<0.1 <0.2				0.5	0.5	0.1 <0.1	0.1 <0.1
M16	2.4	<0.1 <0.1		<0.1	<0.1	<0.1 <0.1	<0.1 <0.1		
M19	5.3	<0.1 <0.1	0.1 <0.1	0.1 <0.1		<0.1 <0.1			

CS - contaminated sample.
<X - none detected at detection limit X

a Presumed pipeline source, based on microbial parameters.
b Possible contamination.
c Probable contamination, excluded from summary tables.
d Wastewater chlorinated at Lubbock treatment plant.
e Run conducted at night.

TABLE P-22. DISTRIBUTION OF PARTICIPANT EXPOSURE MEASURES XAEREM, XDIREM AND FHRSEM IN 1983 IRRIGATION PERIODS

	Irrigation period	
	3 Spring 1983	4 Summer 1983
XAEREM, Index of Extensive Aerosol Exposures		
Minimum	0	0
Maximum	157.4	288.0
XAEREM Levels (XAEREL)		
# None (XAEREM=0)	293(87%)	276(88%)
# Low (0.1≤XAEREM≤10)	22(7%)	21(7%)
# High (XAEREM>10)	20(6%)	18(6%)
XDIREM, Index of Extensive Direct Wastewater Contacts		
Minimum	0	0
Maximum	303.7	438.5
XDIREM Levels (XDIREL)		
# None (XDIREM=0)	316(94%)	290(92%)
# Low (0.1≤XDIREM≤10)	8(2%)	10(3%)
# High (XDIREM>10)	11(3%)	15(5%)
FHRSEM, Average Hours per Week on Hancock Farm		
Minimum	0	0
Maximum	160.8	158.8
FHRSEM Levels (FHRSEL)		
# None (FHRSEM=0)	272(81%)	240(76%)
# Low (0.1≤FHRSEM≤20)	37(12%)	49(16%)
# High (FHRSEM>20)	26(8%)	26(8%)

TABLE P-23. CORRELATION COEFFICIENTS r AMONG LOGARITHMICALLY TRANSFORMED[a] EXPOSURE MEASURES

	Season	AEI	XAEREM	XDIREM	FHRSEM
XAEREM	Spring 1983	0.508			
	Summer 1983	0.610			
XDIREM	Spring 1983	0.365	0.767		
	Summer 1983	0.536	0.901		
FHRSEM	Spring 1983	0.445	0.807	0.593	
	Summer 1983	0.579	0.755	0.630	
TLUBOCK[b]	Spring 1983	-0.058	0.139	0.058	0.167
	Summer 1983	0.005	0.067	0.024	0.162

[a] Natural logarithm (exposure measure + detection limit/10) used to improve the symmetry of each marginal distribution, especially for AEI.

[b] TLUBOCK = hours per week spent in Lubbock; weighted average of activity diary values.

TABLE P-24. DEMOGRAPHIC CHARACTERISTICS OF PARTICIPATING HOUSEHOLDS BASED ON RESPONSES TO THE INITIAL (MAY 1980) AND FINAL (OCTOBER 1983) QUESTIONNAIRE

	Household location by sampling zone						
	Rural 0-0.5 mile	Wilson 0-0.5 mile	Rural 0.5-1 mile	Wilson 0.5-1 mile	Rural 1-2+ miles	Workers >2 miles	Total
1980 #	28	36	14	40	42	3	163
1980 %	17	22	9	25	26	2	100
1983 #	19	21	8	30	27	2	107
1983 %	18	20	7	28	25	2	100

	Race		
	Caucasian	Hispanic	Total
1980 #	133	30	163
1980 %	82	18	100
1983 #	91	16	107
1983 %	85	15	100

	Number of household members									
	1	2	3	4	5	6	7	9	10	Total
1980 #	34	56	26	21	13	6	4	1	2	163
1980 %	21	34	16	13	8	4	2	1	1	100
1983 #	24	40	11	14	9	5	3		1	107
1983 %	22	37	10	13	8	5	3			100

	Education category of head of household						
	NR	0-8	9-11	12	Some college (13-15)	College grad (16-18)	Total
1980 #	4	53	20	52	18	16	163
1980 %	2	33	12	32	11	10	100
1983 #		34	11	36	14	12	107
1983 %		32	10	34	13	11	100

continued...

TABLE P-24. (CONT'D)

	Most educated member of household (1983 only)				
	0-11	12	Some college (13-15)	College grad (16-18)	Total
1983 #	16	34	21	36	107
1983 %	15	32	20	34	100

	Total household income in 1979										
	NR	<5000	5000-7999	8000-9999	10000-14999	15000-19999	20000-29999	>30000	Don't know	Refused	Total
1980 #	1	21	25	14	21	22	24	31	1	3	163
1980 %	1	13	15	9	13	13	15	19	1	2	100
1983 #		13	17	9	14	12	17	23	2		107
1983 %		12	16	8	13	11	16	21	2		100

	Location of households		
	Rural	Wilson	Total
1980 #	86	76	162
1980 %	53	47	100
1983 #	55	51	106
1983 %	52	48	100

	Classification of households by presence of children			
	Child <5	Child 6-17	No children	Total
1980 #	97	42	24	163
1980 %	60	26	15	100
1983 #	69	26	12	107
1983 %	64	24	11	100

continued...

TABLE P-24. (CONT'D)

	Air conditioning system					
	NR	None	Refrig- eration	Evapor cooler	Type unknown	Total
1980 #	3	18	52	46	44	163
1980 %	2	11	32	28	27	100
1983 #		13	52	42		107
1983 %		12	49	39		100

	Source of drinking water				
	NR	Wilson	Canad. river	Private well	Total
1980 #	1	72	4	86	163
1980 %	1	44	2	53	100
1983 #	1	49	3	53	107
1983 %	1	46	3	50	100

	Sewage disposal			
	NR	Septic tank	City system	Total
1980 #	1	91	71	163
1980 %	1	56	44	100
1983 #		59	48	107
1983 %		55	45	100

TABLE P-25. DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS BASED ON RESPONSES TO THE INITIAL[a] (MAY 1980) AND FINAL (OCTOBER 1983) QUESTIONNAIRES

	Race		
	Caucasian	Hispanic	Total
1980 #	337	145	482[a]
1980 %	70	30	100
1983 #	221	85	306
1983 %	72	28	100

	Household location						
	Rural 0 to 0.5 mile	Wilson 0 to 0.5 mile	Rural 0.5 to 1 mile	Wilson 0.5 to 1 mile	Rural 1 to 2+ miles	Workers >2 miles	Total
1980 #	68	117	47	120	122	8	482
1980 %	14	24	10	25	25	2	100
1983 #	44	71	27	84	74	6	306
1983 %	14	23	9	27	24	2	100

	Dwelling location			
	Other	Rural	Wilson	Total
1980 #	5	240	237	482
1980 %	1	50	49	100
1983 #	1	148	155	306
1983 %	0	48	52	100

	Age group (as of June 30, 1982)						
	NR	0-5	6-17	18-44	45-64	65+	Total
1980 #	1	34	118	173	94	62	482
1980 %	0	7	24	36	20	13	100
1983 #		21	79	86	79	41	306
1983 %		7	26	28	26	13	100

	Sex		
	Male	Female	Total
1980 #	237	245	482
1980 %	49	51	100
1983 #	143	163	306
1983 %	47	53	100

continued...

TABLE P-25 (CONT'D)

	Drinks bottled water regularly			
	NR	No	Yes	Total
1980 #	3	416	63	482
1980 %	1	86	13	100
1983 #	2	248	56	306
1983 %	1	81	18	100

	Smokes cigarettes regularly			
	NR	No	Yes	Total
1980 #	1	413	68	482
1980 %	0	86	14	100
1983 #	3	265	38	306
1983 %	1	87	12	100

	Trips to Lubbock per month (1980 only)				
	NR	0-5	6-10	11+	Total
1980 #	7	292	81	102	482
1980 %	1	61	17	21	100

	Hours in Lubbock per trip (1980 only)						
	NR	0-5	6-15	16-25	26-100	>100	Total
1980 #	27	358	89	5	2	1	482
1980 %	5	74	18	1	0	0	100

	Tapwater consumed vs. others your age (1983 only)				
	NR	Less than Average	Average	More than Average	Total
1983 #	4	51	208	43	306
1983 %	1	17	68	14	100

	Contacts per week with 10 or more people (1983 only)						
	NR	Less than once	1-5	6-10	11-15	More than 15	Total
1983 #	3	10	124	92	43	34	306
1983 %	1	3	41	30	14	11	100

	Chews tobacco regularly (1983 only)			
	NR	No	Yes	Total
1983 #	3	281	22	306
1983 %	1	92	7	100

[a] Includes four individuals who only provided an initial blood sample.

TABLE P-26. CROSSTABULATION OF SELECTED HOUSEHOLD VARIABLES BY OVERALL AEROSOL EXPOSURE INDEX LEVEL

Household AEI level for 1982 and 1983	Grouped household size				
	NR	1 person	2-4 people	>5 people	Total
Dropped		9	34	7	50
Low exp	2	2	18	4	26
Med exp		19	32	15	66
Hi exp		4	15	2	21
TOTAL	2	34	99	28	163

Household AEI level for 1982 and 1983	Head of household occupation group				
	NR	Prof + mgr (1or2)	Farmer (9or10)	Other	Total
Dropped	3	4	17	26	50
Low exp		4	14	8	26
Med exp		14	11	41	66
Hi exp		2	17	2	21
TOTAL	3	24	59	77	163

Household AEI level for 1982 and 1983	Education category of head of household						
	NR	0-8	9-11	12	Some college (13-15)	College grad (16-18)	Total
Dropped	4	16	9	15	3	3	50
Low exp		8	2	9	4	3	26
Med exp		25	9	16	9	7	66
Hi exp		4		12	2	3	21
TOTAL	4	53	20	52	18	16	163

continued...

TABLE P-26. (CONT'D)

Household AEI level for 1982 and 1983	Total household income in 1979										
	NR	<5000	5000-7999	8000-9999	10000-14999	15000-19999	20000-29999	>30000	Don't know	Refused	Total
Dropped	1	7	8	4	5	10	5	8	1	1	50
Low exp		1	3	3	6	1	4	8			26
Med exp		12	10	5	7	8	11	11		2	66
Hi exp		1	4	2	3	3	4	4			21
TOTAL	1	21	25	14	21	22	24	31	1	3	163

Household AEI level for 1982 and 1983	Air conditioning system					
	NR	None	Refrig-eration	Evapor cooler	Type unknown	Total
Dropped	3	4		1	42	50
Low exp		3	17	6		26
Med exp		7	29	28	2	66
Hi exp		4	6	11		21
TOTAL	3	18	52	46	44	163

TABLE P-27. CROSSTABULATION OF SELECTED PARTICIPANT[a] VARIABLES BY OVERALL AEROSOL EXPOSURE INDEX LEVEL

Aerosol exposure level	Age group (as of June 30, 1982)						
	NR	0-5	6-17	18-44	45-64	65+	Total
Dropped	1	18	36	86	16	21	178
Low exp		5	25	25	31	11	97
Med exp		8	49	48	38	27	170
Hi exp		3	8	14	9	3	37
Total	1	34	118	173	94	62	482[a]

	Race of respondent		
	Caucasian	Hispanic	Total
Dropped	112	66	178
Low exp	71	26	97
Med exp	120	50	170
Hi exp	34	3	37
Total	337	145	482

	Chews tobacco regularly			
	NR	No	Yes	Total
Dropped	166	11	1	178
Low exp	1	94	2	97
Med exp	10	149	11	170
Hi exp	1	28	8	37
Total	178	282	22	482

	History of chronic illness		
	No	Yes	Total
Dropped	100	78	178
Low exp	42	55	97
Med exp	74	96	170
Hi exp	15	22	37
Total	231	251	482

	Male	Female	Total
Dropped	92	86	178
Low exp	49	48	97
Med exp	75	95	170
Hi exp	21	16	37
Total	237	245	482

continued...

TABLE P-27. (CONT'D)

Aerosol exposure level	Education category of head of household (used as index of socioeconomic status)						
	NR	0-8	9-11	12	Some college (13-15)	College grad (16-18)	Total
Dropped	4	70	27	57	7	13	178
Low exp		37	4	34	13	9	97
Med exp		58	19	45	29	19	170
Hi exp		10		15	6	6	37
Total	4	175	50	151	55	47	482[a]

	Recommended for polio immunization		
	No	Yes	Total
Dropped	136	42	178
Low exp	64	33	97
Med exp	91	79	170
Hi exp	18	19	37
Total	309	173	482

[a] Includes four individuals who only provided an initial blood sample.

TABLE P-28. CROSSTABULATION OF SELECTED DEMOGRAPHIC VARIABLES[a]

	Age group (as of June 30, 1982)						
	NR	0-5	6-17	18-44	45-64	65+	Total
Recommended for polio immunization							
Yes		8	55	54	36	20	173
No		26	63	119	58	42	308
Total		34	118	173	94	62	481
Sex							
Male	1	18	60	88	42	28	237
Female		16	58	85	52	34	245
Total	1	34	118	173	94	62	482[a]
Dwelling location							
Other	1		2	2			5
Rural		19	45	88	54	34	240
Wilson		15	71	83	40	28	237
Total	1	34	118	173	94	62	482
Race of respondent							
Caucasian	1	21	61	122	75	57	337
Hispanic		13	57	51	19	5	145
Total	1	34	118	173	94	62	482

	Race		
Dwelling location	Caucasian	Hispanic	Total
Other	5		5
Rural	214	26	240
Wilson	118	119	237
Total	337	145	482

Dwelling location	History of chronic illness No	Yes	Total
Other	2	3	5
Rural	99	141	240
Wilson	130	107	237
Total	231	251	482

[a] Includes four individuals who only provided an initial blood sample.

TABLE P-29. HEALTH HISTORY OF STUDY PARTICIPANTS[a]

	Age at onset						
Condition	0-5	6-11	12-17	18-30	31-50	51+	Total
Chronic respiratory conditions in study population by age at onset							
Allergies	32	22	8	11	5	13	91
Chronic bronchitis	4	1	1	2	5	3	16
Emphysema					2	5	7
Asthma	14	2	2	4	1	4	27
Tumor or cancer of the lung						1	1
Tumor or cancer of the mouth or throat						1	1
Other				1	2	3	6
Chronic abdominal conditions in study population by age at onset							
Tumor or cancer of							
Stomach				1			1
Intestine							0
Colon							0
Esophagus					1		1
Peptic or duodenal ulcer	1	3	3	10	11	7	35
Ulcerative colitis		1			1	1	3
Diverticulitis					3	6	9
Gall bladder				3	12	10	25
Other	1			4	8	7	20
Chronic cardiovascular conditions in study population by age at onset							
High blood pressure				8	27	40	75
Stroke					1	4	5
Heart attack					2	3	5
Angina					2	3	5
Other		1		1	3	6	11
Other chronic conditions in study population by age at onset							
Skin cancer				3	7	10	20
Leukemia				1			1
Hodgkins							0
Other cancers				2	2	5	9
Arthritis	1	1	1	9	22	44	78
Diabetes		2		1	1	8	12
Anemia	2		1	3		3	9
Immunologic disorder						1	1
Rheumatic fever		3				1	4

continued...

TABLE P-29. (CONT'D)

	Age at onset						
Condition	0-5	6-11	12-17	18-30	31-50	51+	Total
Infectious hepatitis	2	4	3	2	3		14
Serum hepatitis	1	1		1	1		4
Mononuecleosis	1		1		1		3
Other chronic	6	3		3	6	12	30

Blood transfusion (1980 only)

	NR	No	Yes	Don't know	Total
1980 number	1	435	42	4	482[a]
1980 percent	0	90	9	1	100

Hemodialysis (1980 only)

	NR	No	Yes	Total
1980 number	1	479	2	482
1980 percent	0	99	0	100

Close contact of person with tuberculosis (1980 only)

	NR	No	Yes	Total
1980 number	1	467	14	482
1980 percent	0	97	3	100

History of pneumonia (asked only in 1982)

	NR	No	Yes	Total
1982 number	86	362	34	482
1982 percent	18	75	7	100

History of cancer in blood relatives of household adults (1983 only)

	No	Yes	Total
1983 number	106	83	186
1983 percent	56	44	100

[a] Includes four individuals who only provided an initial blood sample.

TABLE P-30. CROPS AND LIVESTOCK

	Crop types (in acres)						
	Total acres farmed	Cotton	Wheat	Oats	Milo	Other	Payment in kind
1980	38045	23885	993	NR	NR	2344	NR
1983	29623	14023	1105	339	2607	1192	2320

	Livestock						
	Cattle	Hogs	Sheep	Fowl	Horses	Other	Total
1980	297	886	175	227	0	0	1585
1983	121	100	51	124	NR	8	404

	Farmland irrigation		
	No	Yes	Total farms
1980	4	67	71
1983	11	25	36

TABLE P-31. COMPARISON OF CHARACTERISTICS: CAUCASIAN PARTICIPANTS VS. HISPANIC PARTICIPANTS

Variable	n	p value	Comment
ACOND	161	0.03	higher proportion of ''yes'' in caucasian HHs
ACSYS	116	0.001	higher proportion of caucasians report ''refrigeration''
ABDOM	477	0.001	higher proportion of caucasians report ''yes''
AGEGRP	477	<0.001	higher proportion of hispanics age 17 or less; caucasians 65+
BOTTLED3	303	0.06	higher proportion of caucasians report ''yes''
CHRONIC	478	<0.001	higher proportion of caucasians report ''yes''
DWATER-B	477	<0.001	higher proportion of hispanics drink ''public'' water
GHSIZE	468	<0.001	higher proportion of hispanics live in HH with 5+; higher proportion of caucasians live in HH of 1
GINCOME	158	0.005	higher proportion of caucasians report $10,000+
HCHILD	475	<0.001	higher proportion of hispanics live in HHs with children
HEART	477	<0.001	higher proportion of caucasians report ''yes''
HOHEDGR	474	<0.001	higher proportion of caucasians report ''college''
HOHOCC	160	0.026	higher proportion of caucasian HHs headed by ''prof. or manager''
LOCATE	478	<0.001	higher proportion of hispanics live in Wilson
OTHERO	477	<0.001	higher proportion of caucasians report ''yes''
RESP	477	<0.001	higher proportion of caucasians report ''yes''
SEX	478		
SMOKE	302		

TABLE P-32. COMPARISON OF CHARACTERISTICS: RURAL PARTICIPANTS VS. WILSON PARTICIPANTS

Variable	n	p value	Comment
ACOND	161		
ACSYS	116		
ABDOM	477		
AGEGRP	477		
BOTTLED3	303		
CHRONIC	478	0.002	higher proportion of ''yes'' in rural
DWATER-B	477	<0.001	''public'' in Wilson, ''private'' in rural
GHSIZE	468	0.007	higher proportion of single and 5+ HHs in Wilson
GINCOME	163	0.007	higher proportion of high income HHs in rural area
HCHILD	478	0.004	higher proportion of HHs with children in Wilson
HEART	477		
HOHEDGR	474	<0.001	higher level of education in rural
HOHOCC	477	<0.001	higher proportion of farmers in rural
OTHERO	477		
RACE	478	<0.001	higher proportion of ''hispanic'' in Wilson
RESP	477		
SEX	478		

TABLE P-33. COMPARISON OF STUDY PARTICIPANT CHARACTERISITICS BY SAMPLING ZONE

Variable	n	p value	Comment
ACOND	163		
ACSYS	116		
ABDOM	477	0.074	higher proportion of ''yes'' in Zones 1 and 3
AGEGRP	477		
BOTTLED3	475	<0.001	higher proportion of ''yes'' in Zone 3; lowest in Zone 4
CHRONIC	478		
DWATER-B	477	<0.001	''public'' in Zones 2 and 4; ''private'' in Zones 1, 3 and 5
GHSIZE	468	0.038	higher proportion of single member HHs in Zones 1 and 4
GINCOME	163	0.008	higher proportion of $30,000 in Zones 1 and 5
HCHILD	478	0.011	higher proportion of HHs without children in Zone 1
HEART	477		
HOHEDGR	474	<0.001	higher proportion of ''college'' in Zone 1
HOHOCC	160	<0.001	higher proportion of ''farmer'' in Zones 1 and 3
OTHERO	477		
RACE	477	<0.001	higher proportion of ''hispanic'' in Zones 2 and 4 (Wilson)
RESP	477	0.016	highest proportion of ''yes'' in Zone 3; lowest in Zone 4
SEX	436		
SMOKE	477	0.082	highest proportion of ''yes'' in Zone 2; lowest in Zone 3

TABLE P-34. COMPARISON OF CHARACTERISTICS: PARTICIPANTS WHO PROVIDED ALL REQUESTED BLOOD SAMPLES VS. THOSE WHO PROVIDED EITHER SOME (4-7) OR FEW (1-3) OF THE REQUESTED SAMPLES

Variable	n	p value	Comment
ACOND	433	<0.001	higher proportion of ''yes'' provided all samples
ACSYS	317	0.046	higher proportion of ''refrigeration'' provided all samples
ABDOM	429		
AGEGRP	435	0.001	higher proportion of ages 45+ provided all samples
BOTTLED3	291		
CHRONIC	436	<0.001	higher proportion of ''yes'' provided all samples
DWATER-B	435	0.016	higher proportion of ''bottled'' and ''public'' provided all samples
GHSIZE	429		
GINCOME	426	0.041	higher proportion of $20,000+ provided all samples
HCHILD	436	<0.001	higher proportion of HHs without children provided all samples
HEART	435	0.01	higher proportion of ''yes'' provided all samples
HOHEDGR	432	0.005	higher proportion of ''college education'' provided all samples
HOHOCC	433	0.002	higher proportion of ''prof. or manager'' provided all samples
LOCATE	436	<0.001	higher proportion of ''Wilson'' provided 4-8 samples
OTHERO	435	0.006	higher proportion of ''yes'' provided all samples
RACE	436	<0.001	higher proportion of ''caucasian'' provided all samples
RESP	435		
SEX	436		
SMOKE	290		
ZONE	436		

TABLE P-35. COMPARISON OF CHARACTERISTICS: SENTINEL POPULATION VS. GENERAL STUDY POPULATION

Variable	n	P value	Comment
ACOND	472		
ACSYS	472	0.093	higher proportion of ''refrigeration'' in sentinel
ABDOM	472		
AGEGRP	472		
BOTTLED3	472	0.095	higher proportion of ''yes'' in sentinel
CHRONIC	472	0.005	higher proportion of ''yes'' in sentinel
DWATER-B	472	<0.001	higher proportion of ''private well'' in sentinel
GHSIZE	472		
GINCOME	472	0.078	higher income in sentinel
HCHILD	472		
HEART	472		
HOHEDGR	472	<0.001	higher education level in sentinel
HOHOCC	472	<0.001	higher proportion of ''prof or manage'' in sentinel
LOCATE	472	<0.001	higher proportion of ''rural'' in sentinel
OTHERO	472		
RACE	472	<0.001	higher proportion of ''caucasian'' in sentinel
RESP	472	0.026	higher proportion of ''yes'' in sentinel
SEX	472		
SMOKE	302	0.019	higher proportion of ''no'' in sentinel
ZONE	472	<0.001	higher proportion of ''Zone 1'' in sentinel

TABLE P-36. DEMOGRAPHIC DIFFERENCES BETWEEN FECAL DONORS AND NONDONORS DURING SUMMER 1982

Variable	n	p value	Comment
GINCOME	478	0.045	higher proportion of fecal donors from low income households
HCHILD	478	0.014	lower proportion of HHs with children age 6-17 were fecal donors
SEX	478	0.063	higher proportion of fecal donors were female

TABLE P-37. DEMOGRAPHIC DIFFERENCES BETWEEN FECAL DONORS AND NONDONORS DURING SPRING 1983

Variable	n	p value	Comment
CHRONIC	478	0.042	higher proportion of fecal donors reported ''yes''
GHSIZE	468	0.001	higher proportion of fecal donors from single member HHs
HCHILD	478	0.081	higher proportion of fecal donors from HHs without children
RACE	478	0.015	higher proportion of fecal donors were caucasian
SMOKE3	302	0.048	higher proportion of fecal donors were nonsmokers?

TABLE P-38. DEMOGRAPHIC DIFFERENCES BETWEEN FECAL DONORS AND NONDONORS DURING SUMMER 1983

Variable	n	p value	Comment
CHRONIC	478	0.042	higher proportion of fecal donors reported ''yes''
GHSIZE	478	0.001	higher proportion of fecal donors from single member HHs
GINCOME	478	0.073	higher proportion of fecal donors reported low income
HCHILD	478	0.072	higher proportion of fecal donors from HHs without children
RACE	478	0.033	higher proportion of fecal donors were caucasian
SMOKE3	302	0.011	higher proportion of fecal donors were nonsmokers

TABLE P-39. DEMOGRAPHIC DIFFERENCES OBSERVED BETWEEN EXPOSURE GROUP SUBPOPULATIONS AND BETWEEN EXPOSURE LEVEL SUBPOPULATIONS DURING SPRING 1982

	Exposure group			Exposure level		
Variable	n	p-value	Comment	n	p-value	Comment
ACOND	377			374	0.076	higher proportion of ''none'' in med exp
ACSYS	314	0.016	higher proportion of hi exp used evaporative cooler	314	0.001	higher proportion of evaporative cooler in hi exp
DWATER-B	377			376	<0.001	higher proportion of ''public'' in med exp
GHSIZE	369	0.092	higher proportion of 1-4 mem HHs in high exposure	369		
GINCOME	368	0.003	higher proportion of $20,000+ in low exp	368	<0.001	higher proportion of $20,000+ in low exp
HOHEDGR	374			374	0.004	higher proportion of ''college'' in hi exp
LOCATE	377	<0.001	higher proportion of Wilson residents in hi exp	377	<0.001	higher proportion of Wilson in med exp
RACE	377	0.078	higher proportion of hispanics in hi exp	377	<0.001	higher proportion of hispanic in med exp
ZONE	377	<0.001	higher proportion of Zones 1 and 2 in hi exp	377	<0.001	higher proportion of Zones 2 and 4 in med; Zone 1 in hi exp

TABLE P-40. DEMOGRAPHIC DIFFERENCES OBSERVED BETWEEN EXPOSURE GROUP SUBPOPULATIONS AND BETWEEN EXPOSURE LEVEL SUBPOPULATIONS DURING SUMMER 1982

	Exposure group			Exposure level		
Variable	n	p-value	Comment	n	p-value	Comment
ACSYS	317	0.024	higher proportion of ''evaporative'' in hi exp	317		
DWATER-B	363			364	0.008	higher proportion of ''public'' in med exp
GHSIZE	354	0.046	lower proportion of 5+ HHs in hi exp	364	0.011	higher proportion of ''2-4'' in hi exp
GINCOME	355			364	0.057	higher proportion of $20,000+ and $10,000 in low exp
HCHILD	364	0.012	lower proportion of HHs with children in hi exp	364		
HOHEDGR	363			361	0.037	higher proportion of ''college'' in hi exp
LOCATE	364			364	<0.001	higher proportion of Wilson in med exp
RACE	364			364	0.003	higher proportion of hispanic in med exp
ZONE	364	<0.001	higher proportion of Zones 1 and 2 in hi exp	364	<0.001	higher proportion of Zones 2 and 4 in med; Zone 1 in hi exp

TABLE P-41. DEMOGRAPHIC DIFFERENCES OBSERVED BETWEEN EXPOSURE GROUP SUBPOPULATIONS AND BETWEEN EXPOSURE LEVEL SUBPOPULATIONS DURING SPRING 1983

	Exposure group			Exposure level		
Variable	n	p-value	Comment	n	p-value	Comment
ACOND	331			331	0.048	higher proportion of ''yes'' in hi exp
ACSYS	309	<0.001	higher proportion of ''evaporative'' in hi exp	309	<0.001	higher proportion of ''evaporative'' in hi exp
DWATER-B	333			333	<0.001	higher proportion of ''public'' in med exp
GHSIZE	323	<0.001	higher proportion of 1-4 HH members in hi exp	323	0.022	higher proportion of 1-4 member HHs in hi exp
GINCOME	333	0.022	higher proportion of $20,000+ in low exp	325	0.001	higher proportion of $20,000+ in lo exp
HCHILD	333	0.025	higher proportion of HHs with no children in hi exp	333	0.083	higher proportion of HHs with no children in hi exp
HOHOCC	332			332	0.037	lower proportion of ''farmer'' in med exp
LOCATE	333	<0.001	higher proportion of Wilson in hi exp	333	<0.001	higher proportion of Wilson in med exp
RACE	333			333	<0.001	higher proportion of hispanic in med exp
ZONE	333	<0.001	higher proportion of Zones 1 and 2 in hi exp	333	<0.001	higher proportion of Zones 2 and 4 in med; Zone 1 in hi exp

TABLE P-42. DEMOGRAPHIC DIFFERENCES OBSERVED BETWEEN EXPOSURE GROUP SUBPOPULATIONS AND BETWEEN EXPOSURE LEVEL SUBPOPULATIONS DURING SUMMER 1983

	Exposure group			Exposure level		
Variable	n	p-value	Comment	n	p-value	Comment
ACSYS	308	<0.001	higher proportion of ''evaporative'' in hi exp	308	<0.001	higher proportion of ''evaporative'' in hi exp
DWATER-B	313			313	<0.001	higher proportion of ''public'' in med exp
GHSIZE	303	0.056	higher proportion of 1-4 HH members in hi exp	303		
LOCATE	313			313	<0.001	higher proportion of wilson in med exp
ZONE	313	<0.001	higher proportion of Zones 1 and 2 in hi exp	313	<0.001	higher proportion of Zones 2 and 4 in med; Zone 1 in hi exp

TABLE P-43. DEMOGRAPHIC DIFFERENCES OBSERVED BETWEEN EXPOSURE GROUP SUBPOPULATIONS AND BETWEEN EXPOSURE LEVEL SUBPOPULATIONS DURING 1982

	Exposure group			Exposure level		
Variable	n	p-value	Comment	n	p-value	Comment
ACSYS	312	0.005	higher proportion of ''evaporative'' in hi exp	312	0.018	higher proportion of ''evaporative'' in hi exp
CONTACT	293	0.037	higher proportion of 6+ contacts in low exp	293		
DWATER	358			358	<0.001	higher proportion of ''public'' in med exp
GHSIZE	359	0.057	higher proportion of 5+ in low exp	359	0.003	higher proportion of ''2-4'' in hi exp
GINCOME	350			350	0.037	higher proportion of $20,000+ in low exp
HCHILD	359	0.53	higher proportion of HH's with no children in hi exp	359		
HOHEDGR	356			356	0.039	higher proportion of hi exp reported college education
HOHOCC	357	0.014	higher proportion of farmer in hi exp	357	<0.001	higher proportion of farmers in hi,low exp
LOCATE	359			359	<0.001	higher proportion of Wilson in med exp
RACE	359			359	0.004	lower proportion of hispanic in hi exp
SMOKE3	293			293	0.91	higher proportion of smokers in med exp
ZONE	385	<0.001		359	<0.001	higher proportion of Zones 1 and 2 in hi exp; Zones 4 and 5 in low exp

TABLE P-44. DEMOGRAPHIC DIFFERENCES OBSERVED BETWEEN EXPOSURE GROUP SUBPOPULATIONS AND BETWEEN EXPOSURE LEVEL SUBPOPULATIONS DURING 1983

	Exposure group			Exposure level		
Variable	n	p-value	Comment	n	p-value	Comment
ACSYS	308	<0.001	higher proportion of ''evaporative'' in hi exp	309	<0.001	higher proportion of ''evaporative'' in hi exp; ''refrigeration'' in low
CHRONIC	313	0.089	higher proportion of ''yes'' in hi exp	314		
DWATER	313			314	<0.001	higher proportion of ''public'' in med exp
GHSIZE	313	0.085	higher proportion of 5+ in low exp	314		
HOHOCC	313	<0.001	higher proportion of farmer in hi exp	314	<0.001	higher proportion of farmer in hi exp
LOCATE	313			314	<0.001	higher proportion of Wilson in med exp
SMOKE3	301			301	0.066	higher proportion of smokers in med exp
ZONE	313	<0.001	higher proportion of Zones 1 and 2 in hi exp; Zones 4 and 5 in low	314	<0.001	higher proportion of Zones 1 and 2 hi exp; Zones 4 and 5 in low exp

TABLE P-45. FREQUENCY DISTRIBUTION OF TITERS BY AGENT AND COLLECTION PERIOD

Agent Titer	Jun 80 (012-016)	Dec 80 (025-111)	Jun 81 (112-120)	Jan 82 (201-206)	Jun 82 (212-218)	Dec 82 (225-305)	Jun 83 (312-314)	Oct 83 (320-323)
Adenovirus 3								
<10	57%	48%	30%	51%	52%	51%	48%	50%
10	14%	19%	15%	14%	30%	15%	12%	20%
20	13%	14%	20%	17%	7%	14%	16%	15%
40	10%	13%	5%	9%	11%	11%	4%	9%
80	5%	3%	30%	5%	0%	7%	8%	5%
160	1%	1%	0%	3%	0%	2%	8%	0%
320	0%	1%	0%	1%	0%	1%	4%	0%
640	0%	0%	0%	0%	0%	0%	0%	0%
	N=214	N=69	N=20	N=276	N=27	N=303	N=26	N=266
Adenovirus 5								
<10	53%	40%	38%	46%	30%	46%	27%	44%
10	9%	18%	13%	12%	10%	11%	5%	11%
20	16%	19%	6%	17%	7%	17%	11%	18%
40	13%	6%	19%	11%	17%	15%	22%	15%
80	8%	12%	19%	9%	20%	6%	16%	8%
160	3%	3%	6%	4%	10%	3%	16%	4%
320	0%	1%	0%	0%	5%	1%	3%	0%
640	0%	0%	0%	0%	0%	0%	0%	0%
	N=216	N=68	N=16	N=279	N=40	N=302	N=37	N=266
Adenovirus 7								
<10	78%	77%	50%	72%	70%	33%	81%	86%
10	17%	15%	25%	17%	21%	67%	19%	11%
20	5%	6%	19%	9%	8%	0%	0%	3%
40	0%	1%	6%	3%	2%	0%	0%	0%
80	0%	0%	0%	0%	0%	0%	0%	0%
	N=236	N=79	N=16	N=305	N=304	N=3	N=21	N=266
Coxsackievirus A9								
<10	43%	17%	33%	27%				
10	9%	14%	18%	14%				
20	15%	18%	18%	12%				
40	15%	24%	9%	16%				
80	11%	4%	18%	13%				
160	6%	4%	9%	5%				
320	0%	4%	5%	2%				
640	0%	0%	0%	0%				
	N=245	N=50	N=11	N=306				
Coxsackievirus B2								
<10	21%	21%	26%	23%	24%	25%	100	0%
10	11%	19%	11%	10%	9%	10%	0%	0%
20	20%	13%	21%	20%	9%	17%	0%	0%
40	23%	24%	5%	20%	9%	20%	0%	0%

continued...

TABLE P-45 (CONT'D)

Agent Titer	Jun 80 (012-016)	Dec 80 (025-111)	Jun 81 (112-120)	Jan 82 (201-206)	Jun 82 (212-218)	Dec 82 (225-305)	Jun 83 (312-314)	Oct 83 (320-323)
Coxsackievirus B2 (Cont'd)								
80	15%	13%	26%	16%	35%	17%	0%	100%
160	6%	11%	5%	10%	12%	8%	0%	0%
320	3%	0%	5%	2%	3%	3%	0%	0%
640	1%	0%	0%	0%	0%	1%	0%	0%
	N=219	N=72	N=19	N=284	N=34	N=303	N=1	N=1
Coxsackievirus B3								
<10	1%	0%	0%	1%				
10	3%	0%	0%	3%				
20	16%	16%	0%	10%				
40	25%	26%	0%	23%				
80	27%	29%	50%	32%				
160	20%	16%	0%	19%				
320	4%	3%	50%	6%				
640	5%	11%	0%	7%				
	N=113	N=38	N=2	N=153				
Coxsackievirus B4								
<10	27%	24%	15%	22%	41%	25%	100%	50%
10	14%	14%	15%	11%	3%	12%	0%	0%
20	22%	17%	8%	19%	13%	14%	0%	0%
40	15%	29%	23%	24%	10%	18%	0%	0%
80	16%	9%	23%	14%	23%	20%	0%	0%
160	4%	6%	15%	8%	8%	8%	0%	50%
320	2%	2%	0%	2%	3%	3%	0%	0%
640	0%	0%	0%	0%	0%	0%	0%	0%
	N=220	N=66	N=13	N=284	N=39	N=303	N=1	N=2
Coxsackievirus B5								
<10	68%	49%	47%	64%	51%	61%	46%	52%
10	16%	26%	18%	14%	19%	13%	14%	18%
20	7%	14%	12%	15%	16%	13%	14%	14%
40	8%	9%	0%	6%	9%	9%	9%	9%
80	1%	1%	12%	2%	4%	4%	11%	2%
160	0%	0%	12%	0%	0%	0%	3%	2%
320	0%	0%	0%	0%	0%	0%	3%	1%
640	0%	0%	0%	0%	0%	0%	0%	0%
	N=238	N=69	N=17	N=307	N=303	N=303	N=35	N=266
Echovirus 1								
<10	90%	86%	91%	84%	88%	100%	90%	92%
10	8%	11%	9%	11%	7%	0%	10%	5%
20	2%	3%	0%	3%	3%	0%	0%	2%
40	0%	0%	0%	1%	1%	0%	0%	1%
80	0%	0%	0%	1%	1%	0%	0%	0%
160	0%	0%	0%	0%	0%	0%	0%	0%
	N=236	N=75	N=11	N=307	N=304	N=1	N=21	N=266

continued...

TABLE P-45. (CONT'D)

Agent Titer	Jun 80 (012-016)	Dec 80 (025-111)	Jun 81 (112-120)	Jan 82 (201-206)	Jun 82 (212-218)	Dec 82 (225-305)	Jun 83 (312-314)	Oct 83 (320-323)
Echovirus 3								
<10	78%	64%	38%	71%	43%	70%	44%	54%
10	12%	11%	13%	12%	18%	12%	15%	20%
20	7%	15%	6%	6%	14%	7%	15%	11%
40	3%	8%	25%	7%	14%	5%	10%	8%
80	0%	2%	13%	3%	7%	4%	8%	5%
160	0%	0%	0%	1%	4%	2%	3%	2%
320	0%	0%	6%	0%	0%	0%	3%	1%
640	0%	2%	0%	0%	0%	0%	3%	1%
	N=214	N=66	N=16	N=276	N=28	N=303	N=39	N=266
Echovirus 5								
<10	72%	67%	44%	69%	66%		76%	81%
10	13%	10%	0%	13%	15%		5%	11%
20	9%	12%	0%	10%	7%		10%	5%
40	4%	3%	11%	3%	7%		10%	1%
80	2%	2%	22%	4%	4%		0%	1%
160	1%	2%	0%	1%	1%		0%	0%
320	0%	3%	22%	1%	0%		0%	0%
	N=223	N=58	N=9	N=279	N=302		N=21	N=263
Echovirus 9								
<10	59%	46%	39%	55%	63%	75%	48%	59%
10	16%	13%	33%	12%	16%	25%	13%	16%
20	11%	14%	6%	11%	13%	0%	17%	12%
40	8%	18%	6%	12%	5%	0%	4%	9%
80	5%	6%	0%	6%	3%	0%	13%	3%
160	2%	3%	6%	3%	0%	0%	4%	2%
320	0%	0%	11%	1%	0%	0%	0%	0%
	N=237	N=71	N=18	N=306	N=302	N=4	N=23	N=263
Echovirus 11								
<10	64%	48%	29%	59%	46%	51%	51%	55%
10	20%	22%	24%	17%	14%	19%	20%	21%
20	8%	9%	14%	12%	19%	17%	17%	14%
40	5%	14%	19%	7%	12%	7%	6%	6%
80	2%	6%	5%	3%	4%	3%	6%	2%
160	1%	0%	5%	1%	2%	3%	0%	1%
320	0%	1%	5%	1%	4%	0%	0%	0%
	N=241	N=69	N=21	N=309	N=57	N=300	N=35	N=269
Echovirus 17								
<10	87%	74%	82%	83%	75%	82%	74%	81%
10	8%	13%	9%	10%	8%	11%	9%	10%
20	3%	7%	0%	5%	0%	5%	0%	6%
40	0%	2%	0%	2%	4%	1%	0%	2%
80	1%	5%	0%	1%	8%	1%	9%	1%
160	0%	0%	9%	0%	4%	0%	9%	1%
	N=213	N=62	N=11	N=274	N=25	N=303	N=23	N=266

continued...

TABLE P-45. (CONT'D)

Agent Titer	Jun 80 (012-016)	Dec 80 (025-111)	Jun 81 (112-120)	Jan 82 (201-206)	Jun 82 (212-218)	Dec 82 (225-305)	Jun 83 (312-314)	Oct 83 (320-323)
Echovirus 19								
<10	82%	81%	54%	79%	52%	77%	100%	91%
10	11%	14%	8%	12%	26%	13%	0%	6%
20	5%	3%	15%	6%	17%	7%	0%	2%
40	1%	2%	8%	2%	4%	2%	0%	0%
80	0%	0%	8%	1%	0%	1%	0%	0%
160	0%	0%	8%	0%	0%	1%	0%	0%
320	0%	0%	0%	0%	0%	0%	0%	0%
	N=211	N=63	N=13	N=271	N=23	N=303	N=21	N=266
Echovirus 20								
<10	82%	84%	77%	83%	67%	79%	53%	67%
10	11%	13%	0%	9%	15%	13%	31%	20%
20	5%	2%	15%	6%	7%	4%	16%	10%
40	1%	0%	0%	2%	7%	4%	0%	3%
80	0%	0%	8%	0%	4%	0%	0%	1%
160	0%	0%	0%	0%	0%	0%	0%	0%
640	0%	2%	0%	0%	0%	0%	0%	0%
	N=217	N=64	N=13	N=277	N=27	N=303	N=32	N=266
Echovirus 24								
<10	89%	81%	64%	79%	52%	74%	72%	84%
10	5%	14%	7%	13%	12%	16%	7%	7%
20	5%	2%	14%	4%	20%	6%	10%	5%
40	1%	2%	0%	2%	4%	4%	3%	2%
80	0%	2%	0%	2%	12%	1%	7%	1%
160	0%	0%	7%	0%	0%	0%	0%	1%
320	0%	0%	7%	0%	0%	0%	0%	0%
640	0%	0%	0%	0%	0%	0%	0%	0%
	N=213	N=64	N=14	N=272	N=25	N=303	N=29	N=266
E. histolytica								
<64	99%			99%			99%	
64	1%			1%			0%	
128	0%			1%			1%	
	N=189			N=189			N=189	
Hepatitis A Virus								
neg	58%	72%	95%	88%	94%	92%	90%	89%
pos	42%	28%	5%	12%	6%	8%	10%	11%
	N=313	N=275	N=169	N=198	N=174	N=178	N=165	N=160
Influenza A								
<4	14%		8%		13%		16%	
4	30%		25%		29%		20%	
8	33%		35%		30%		26%	
16	15%		22%		21%		25%	

continued...

TABLE P-45. (CONT'D)

Agent Titer	Jun 80 (012-016)	Dec 80 (025-111)	Jun 81 (112-120)	Jan 82 (201-206)	Jun 82 (212-218)	Dec 82 (225-305)	Jun 83 (312-314)	Oct 83 (320-323)
Influenza A (Cont'd)								
32	6%		6%		6%		9%	
64	1%		4%		0%		4%	
	N=194		N=251		N=278		N=257	
Legionella								
<64			47%		47%		47%	
64			16%		15%		17%	
128			15%		15%		20%	
256			22%		23%		17%	
			N=269		N=297		N=266	
Norwalk Virus								
<50	11%			14%			51%	
50	16%			5%			11%	
100	16%			23%			6%	
200	16%			18%			6%	
400	11%			14%			14%	
800	11%			9%			3%	
1600	11%			9%			6%	
3200	0%			0%			3%	
6400	11%			5%			3%	
	N=19			N=21			N=36	
Poliovirus 1								
<4	10%	16%	0%	6%	7%	17%		
4	19%	17%	10%	12%	13%	33%		
8	23%	26%	20%	21%	19%	17%		
16	20%	18%	40%	22%	21%	33%		
32	15%	12%	10%	16%	23%	0%		
64	9%	7%	10%	13%	8%	0%		
128	2%	3%	0%	4%	4%	0%		
256	2%	1%	10%	4%	3%	0%		
	N=204	N=311	N=10	N=253	N=307	N=6		
Poliovirus 2								
<4	9%	13%	9%	7%	9%	0%		
4	16%	16%	18%	16%	12%	17%		
8	26%	27%	27%	17%	20%	33%		
16	25%	22%	18%	20%	25%	17%		
32	12%	12%	9%	18%	17%	33%		
64	8%	6%	9%	11%	9%	0%		
128	3%	2%	9%	6%	4%	0%		
256	0%	1%	0%	3%	4%	0%		
	N=210	N=312	N=11	N=250	N=306	N=6		

continued...

TABLE P-45. (CONT'D)

Agent Titer	Jun 80 (012-016)	Dec 80 (025-111)	Jun 81 (112-120)	Jan 82 (201-206)	Jun 82 (212-218)	Dec 82 (225-305)	Jun 83 (312-314)	Oct 83 (320-323)
Poliovirus 3								
<4	37%	41%	40%	20%	27%	16%		
4	26%	24%	10%	22%	21%	50%		
8	17%	14%	10%	21%	17%	17%		
16	8%	10%	20%	12%	16%	17%		
32	9%	7%	10%	10%	9%	0%		
64	2%	2%	0%	7%	5%	0%		
128	1%	0%	10%	5%	4%	0%		
256	0%	0%	0%	3%	2%	0%		
	N=211	N=311	N=10	N=249	N=306	N=6		
Reovirus 1								
<8	70%	47%	50%	58%	65%	52%	63%	
8	11%	22%	0%	12%	11%	18%	17%	
16	6%	7%	25%	7%	7%	14%	7%	
32	6%	5%	8%	10%	7%	10%	7%	
64	4%	12%	17%	6%	6%	3%	4%	
128	1%	3%	0%	3%	3%	2%	1%	
256	0%	1%	0%	1%	1%	1%	1%	
512	1%	3%	0%	2%	0%	0%	0%	
	N=235	N=74	N=12	N=307	N=308	N=300	N=251	
Reovirus 2								
<8	42%	44%	33%	33%	41%	38%	45%	
8	23%	19%	25%	17%	15%	22%	20%	
16	12%	14%	25%	18%	16%	18%	18%	
32	14%	14%	8%	17%	17%	15%	11%	
64	5%	7%	8%	9%	8%	7%	6%	
128	1%	3%	0%	5%	3%	0%	0%	
256	1%	0%	0%	1%	0%	0%	0%	
512	0%	0%	0%	0%	0%	0%	0%	
	N=236	N=73	N=12	N=307	N=308	N=299	N=251	
Rotavirus								
<4	11%	5%	3%	2%	12%	9%	13%	4%
4	7%	0%	6%	5%	10%	6%	2%	2%
8	4%	3%	6%	0%	4%	4%	4%	7%
16	7%	10%	15%	14%	13%	15%	17%	24%
32	18%	28%	18%	23%	17%	23%	28%	29%
64	29%	21%	33%	30%	19%	32%	21%	18%
128	21%	23%	12%	14%	19%	8%	9%	11%
256	4%	3%	6%	12%	6%	4%	6%	4%
512	0%	8%	0%	0%	0%	0%	0%	0%
	N=28	N=39	N=33	N=43	N=52	N=53	N=47	N=45

TABLE P-46. PREVALENCE OF ANTIBODY BY AGENT AND AGE GROUP

Presence of antibody	Age group					Total	
	0-5	6-17	18-44	45-64	65+	N	%
Adenovirus 3							
Positive	31%	32%	53%	53%	36%	135	44%
Negative	69%	68%	47%	47%	64%	171	56%
Total tested	13	81	93	77	42	306	
Adenovirus 5							
Positive	46%	54%	42%	52%	57%	152	50%
Negative	54%	46%	58%	48%	43%	151	50%
Total tested	13	81	92	75	42%	303	
Adenovirus 7							
Positive	8%	7%	29%	29%	27%	74	22%
Negative	92%	93%	71%	71%	73%	256	78%
Total tested	13	84	106	79	48	330	
Coxsackievirus B2							
Positive	27%	67%	77%	90%	90%	239	78%
Negative	73%	33%	23%	10%	10%	69	22%
Total tested	11	83	94	78	42	308	
Coxsackievirus B4							
Positive	17%	66%	74%	81%	77%	224	72%
Negative	83%	34%	26%	19%	23%	89	28%
Total tested	12	85	96	77	43	313	
Coxsackievirus B5							
Positive	13%	42%	43%	23%	31%	121	35%
Negative	87%	58%	57%	77%	69%	223	65%
Total tested	15	91	111	78	49	344	
Echovirus 1							
Positive	0%	2%	9%	15%	26%	36	11%
Negative	100%	98%	91%	85%	74%	296	89%
Total tested	13	86	108	78	47	332	
Echovirus 3							
Positive	38%	35%	26%	21%	24%	83	27%
Negative	62%	65%	74%	79%	76%	222	73%
Total tested	13	80	94	76	42	305	
Echovirus 5							
Positive	0%	17%	30%	35%	36%	91	28%
Negative	100%	83%	70%	65%	64%	238	72%
Total tested	12	87	105	78	47	329	
Echovirus 9							
Positive	25%	44%	46%	41%	39%	141	42%
Negative	75%	56%	54%	59%	61%	193	58%
Total tested	12	87	107	79	49	334	

continued...

TABLE P-46. (CONT'D)

Presence of antibody	Age group					Total	
	0-5	6-17	18-44	45-64	65+	N	%
Echovirus 11							
Positive	31%	40%	38%	42%	48%	138	40%
Negative	69%	60%	62%	58%	52%	203	60%
Total tested	13	90	111	79	48	341	
Echovirus 17							
Positive	8%	7%	12%	25%	19%	45	15%
Negative	92%	93%	88%	75%	81%	259	85%
Total tested	13	82	91	76	42	304	
Echovirus 19							
Positive	15%	4%	16%	23%	47%	56	19%
Negative	85%	96%	84%	77%	52%	245	81%
Total tested	13	81	92	75	40	301	
Echovirus 20							
Positive	8%	5%	19%	22%	22%	48	16%
Negative	92%	95%	81%	78%	78%	254	84%
Total tested	13	81	93	74	41	302	
Echovirus 24							
Positive	23%	9%	13%	20%	17%	44	15%
Negative	77%	91%	87%	80%	83%	258	85%
Total tested	13	80	92	75	42	302	
Hepatitis A							
Positive	0%	15%	30%	65%	98%	178	42%
Negative	100%	85%	70%	35%	2%	248	58%
Total tested	23	104	151	89	59	426	
Influenza A							
Positive	14%	68%	68%	65%	69%	166	66%
Negative	86%	32%	32%	35%	31%	86	34%
Total tested	7	56	82	68	39	252	
Legionella							
Positive	46%	56%	62%	49%	52%	154	55%
Negative	54%	44%	38%	51%	48%	126	45%
Total tested	13	73	77	75	42	280	
Poliovirus 1							
Positive	88%	86%	88%	92%	85%	341	88%
Negative	12%	14%	12%	8%	15%	46	12%
Total tested	17	103	127	85	55	387	
Poliovirus 2							
Positive	100%	93%	86%	89%	76%	340	88%
Negative	0%	7%	14%	11%	24%	47	12%
Total tested	17	103	127	85	55	387	

continued...

TABLE P-46. (CONT'D)

Presence of antibody	Age group 0-5	6-17	18-44	45-64	65+	Total N	Total %
Poliovirus 3							
Positive	59%	44%	67%	73%	64%	237	61%
Negative	41%	56%	33%	27%	36%	150	39%
Total tested	17	105	127	85	55	387	
Reovirus 1							
Positive	29%	24%	40%	43%	35%	120	35%
Negative	71%	76%	60%	57%	65%	219	65%
Total tested	14	89	109	79	48	339	
Reovirus 2							
Positive	31%	47%	60%	61%	75%	195	58%
Negative	69%	53%	40%	39%	25%	143	42%
Total tested	13	89	109	79	48	338	
Rotavirus							
Positive	57%	94%	86%	100%	0%	46	82%
Negative	43%	6%	14%	0%	100%	10	18%
Total tested	14	31	7	3	1	56	

TABLE P-47. INFECTION INCIDENCE DENSITY RATES FOR WASTEWATER AEROSOL EXPOSURE LEVELS BY AGENT AND TIME INTERVAL

(Number of infection events indicated in parentheses)
[When different than number of infection events, number of infected individuals indicated in brackets]

Agent Interval	Low exp level (AEI<1)	Med exp level (1<AEI<5)	High exp level (AEI>5)
Adenovirus 3			
0-Baseline	2.07 (2)	11.40 (11)	0.00 (0)
1-Spring 1982	0.00 (0)	0.00 (0)	0.00 (0)
2-Summer 1982	0.00 (0)	1.27 (1)	0.00 (0)
3-Spring 1983	0.00 (0)	1.33 (1)	0.00 (0)
4-Summer 1983	0.00 (0)	0.00 (0)	0.00 (0)
5-1982	1.19 (1)	3.68 (6)	0.00 (0)
6-1983	0.00 (0)	0.81 (1)	0.00 (0)
7-Irrigation	0.57 (1)	1.91 (7)	0.00 (0)
Adenovirus 5			
0-Baseline	3.16 (3)	5.27 (5)	0.00 (0)
1-Spring 1982	3.53 (1)	2.80 (2)	0.00 (0)
2-Summer 1982	2.18 (1)	1.33 (1)	0.00 (0)
3-Spring 1983	0.00 (0)	2.75 (2)	0.00 (0)
4-Summer 1983	0.00 (0)	0.79 (1)	0.00 (0)
5-1982	2.35 (2)	3.87 (6)	0.00 (0)
6-1983	0.00 (0)	2.55 (3)	3.17 (1)
7-Irrigation	1.15 (2)	2.58 (9)	1.17 (1)
Adenovirus 7			
0-Baseline	0.84 (1)	2.51 (3)	3.38 (2)
1-Spring 1982	0.00 (0)	0.00 (0)	0.00 (0)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)
3-Spring 1983	0.00 (0)	0.00 (0)	0.00 (0)
4-Summer 1983	0.00 (0)	0.00 (0)	0.00 (0)
5-1982	0.00 (0)	0.00 (0)	0.00 (0)
6-1983	0.00 (0)	0.00 (0)	0.00 (0)
7-Irrigation	0.00 (0)	0.00 (0)	0.00 (0)
Coxsackievirus B2			
0-Baseline	7.14 (7)	5.10 (5)	3.32 (2)
1-Spring 1982	0.00 (0)	1.34 (1)	0.00 (0)
2-Summer 1982	0.00 (0)	1.33 (1)	0.00 (0)
3-Spring 1983			
4-Summer 1983			
5-1982	0.00 (0)	4.34 (7)	5.80 (2)
6-1983			
7-Irrigation	0.00 (0)	4.51 (7)	5.80 (2)

continued...

TABLE P-47. (CONT'D)

Agent Interval	Low exp level (AEI<1)	Med exp level (1<AEI<5)	High exp level (AEI>5)
Coxsackievirus B4			
0-Baseline	5.07 (5)	11.15 (11)	0.00 (0)
1-Spring 1982	0.00 (0)	2.64 (2)	6.03 (1)
2-Summer 1982	2.38 (1)	2.57 (2)	11.86 (2)
3-Spring 1983			
4-Summer 1983			
5-1982	8.22 (7)	4.83 (8)	14.49 (5)[4][a]
6-1983			
7-Irrigation	7.93 (6)	5.63 (9)	13.91 (5)[b]
Coxsackievirus B5			
0-Baseline	0.82 (1)	6.57 (8)	3.44 (2)
1-Spring 1982	3.45 (1)	4.08 (3)	0.00 (0)
2-Summer 1982	0.00 (0)	2.52 (2)	10.89 (2)
3-Spring 1983	0.00 (0)	1.34 (1)	0.00 (0)
4-Summer 1983	2.76 (2)	4.59 (6)	0.00 (0)
5-1982	1.16 (1)	2.56 (4)	9.19 (3)
6-1983	3.62 (2)	4.90 (6)	3.21 (1)
7-Irrigation	1.67 (3)	3.62 (13)	2.28 (2)
Echovirus 1			
0-Baseline	0.85 (1)	5.11 (6)	0.00 (0)
1-Spring 1982	3.24 (1)	0.00 (0)	0.00 (0)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)
3-Spring 1983	0.00 (0)	0.00 (0)	0.00 (0)
4-Summer 1983	0.00 (0)	0.00 (0)	0.00 (0)
5-1982	1.16 (1)	0.00 (0)	0.00 (0)
6-1983	0.00 (0)	0.00 (0)	0.00 (0)
7-Irrigation	0.57 (1)	0.00 (0)	0.00 (0)
Echovirus 3			
0-Baseline	8.29 (8)	4.15 (4)	1.80 (1)
1-Spring 1982	0.00 (0)	0.00 (0)	0.00 (0)
2-Summer 1982	0.00 (0)	3.81 (3)	0.00 (0)
3-Spring 1983	6.79 (2)	1.36 (1)	0.00 (0)
4-Summer 1983	1.41 (1)	6.19 (8)	5.77 (2)
5-1982	4.70 (4)	2.51 (4)	3.15 (1)
6-1983	5.61 (3)	9.00 (11)	12.70 (4)
7-Irrigation	3.98 (7)	4.19 (15)	5.75 (5)
Echovirus 5			
0-Baseline	0.96 (1)	0.96 (1)	1.82 (1)
1-Spring 1982	0.00 (0)	1.46 (1)	0.00 (0)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)

continued...

TABLE P-47. (CONT'D)

Agent group Interval	Low exp level (AEI<1)	Med exp level (1<AEI<5)	High exp level (AEI>5)
Echovirus 5 (Cont'd)			
3-Spring 1983	0.00 (0)	0.00 (0)	0.00 (0)
4-Summer 1983	0.00 (0)	0.00 (0)	0.00 (0)
5-1982	0.00 (0)	0.66 (1)	0.00 (0)
6-1983	0.00 (0)	0.00 (0)	0.00 (0)
7-Irrigation	0.00 (0)	0.28 (1)	0.00 (0)
Echovirus 9			
0-Baseline	1.64 (2)	4.11 (5)	3.44 (2)
1-Spring 1982	0.00 (0)	0.00 (0)	0.00 (0)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)
3-Spring 1983	3.37 (1)	0.00 (0)	0.00 (0)
4-Summer 1983	1.44 (1)	0.00 (0)	0.00 (0)
5-1982	0.00 (0)	0.00 (0)	0.00 (0)
6-1983	3.84 (2)	0.00 (0)	0.00 (0)
7-Irrigation	1.19 (2)	0.00 (0)	0.00 (0)
Echovirus 11			
0-Baseline	5.85 (7)	6.69 (8)	3.47 (2)
1-Spring 1982	6.56 (2)	2.58 (2)	0.00 (0)
2-Summer 1982	4.36 (2)	3.80 (3)	12.11 (2)
3-Spring 1983	0.00 (0)	0.00 (0)	0.00 (0)
4-Summer 1983	1.44 (1)	3.04 (4)	2.81 (1)
5-1982	5.75 (5)	4.85 (8)	18.37 (6)[c]
6-1983	5.43 (3)	4.03 (5)	6.19 (2)
7-Irrigation	4.48 (8)	3.79 (14)	7.91 (7)
Echovirus 17			
0-Baseline	1.05 (1)	1.05 (1)	0.00 (0)
1-Spring 1982	0.00 (0)	1.32 (1)	0.00 (0)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)
3-Spring 1983	0.00 (0)	0.00 (0)	0.00 (0)
4-Summer 1983	0.00 (0)	0.00 (0)	0.00 (0)
5-1982	0.00 (0)	0.62 (1)	0.00 (0)
6-1983	0.00 (0)	1.63 (2)	0.00 (0)
7-Irrigation	0.00 (0)	0.83 (3)	0.00 (0)
Echovirus 19			
0-Baseline	0.00 (0)	3.21 (3)	0.00 (0)
1-Spring 1982	0.00 (0)	0.00 (0)	0.00 (0)
2-Summer 1982	0.00 (0)	0.00 (0)	5.93 (1)
3-Spring 1983	0.00 (0)	0.00 (0)	0.00 (0)
4-Summer 1983	0.00 (0)	0.00 (0)	0.00 (0)
5-1982	0.00 (0)	1.24 (2)	3.24 (1)
6-1983	0.00 (0)	0.82 (1)	0.00 (0)
7-Irrigation	0.00 (0)	0.83 (3)	1.18 (1)

continued...

TABLE P-47. (CONT'D)

Agent Interval	Low exp level (AEI<1)	Med exp level (1<AEI<5)	High exp level (AEI>5)
Echovirus 20			
0-Baseline	1.05 (1)	4.19 (4)	0.00 (0)
1-Spring 1982	0.00 (0)	1.33 (1)	0.00 (0)
2-Summer 1982	0.00 (0)	1.28 (1)	0.00 (0)
3-Spring 1983	0.00 (0)	0.00 (0)	0.00 (0)
4-Summer 1983	0.00 (0)	3.14 (4)	5.62 (2)
5-1982	0.00 (0)	1.27 (2)	0.00 (0)
6-1983	3.81 (2)	4.19 (5)	6.35 (2)
7-Irrigation	1.18 (2)	1.97 (7)	2.31 (2)
Echovirus 24			
0-Baseline	2.15 (2)	6.46 (5)	1.80 (1)
1-Spring 1982	3.81 (1)	0.00 (0)	6.71 (1)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)
3-Spring 1983	3.51 (1)	2.69 (2)	5.27 (1)
4-Summer 1983	0.00 (0)	3.83 (5)	5.77 (2)
5-1982	5.02 (4)	1.25 (2)	3.15 (1)
6-1983	1.97 (1)	6.51 (8)[6]	9.77 (3)
7-Irrigation	2.98 (5)	2.77 (10)	4.66 (4)
Influenza A			
0-Baseline	3.24 (3)	12.96 (12)	7.34 (4)
1-Spring 1982	3.69 (1)	8.47 (5)	0.00 (0)
2-Summer 1982			
3-Spring 1983	31.10 (9)	29.82 (22)	21.47 (4)
4-Summer 1983			
7-Irrigation	10.33 (10)	13.29 (27)	8.04 (4)
Legionella			
1-Spring 1982	0.00 (0)	0.54 (1)	2.41 (1)
2-Summer 1982	1.18 (1)	1.28 (2)	0.00 (0)
7-Irrigation	0.59 (1)	0.87 (3)	1.29 (1)
Poliovirus 1[d]			
0-Baseline	2.17 (1)	4.33 (2)	0.00 (0)
1-Spring 1982	0.00 (0)	6.10 (3)	30.45 (3)
Poliovirus 2[d]			
0-Baseline	0.00 (0)	2.24 (1)	0.00 (0)
1-Spring 1982	5.52 (1)	0.00 (0)	10.15 (1)
Poliovirus 3[d]			
0-Baseline	0.00 (0)	0.00 (0)	0.00 (0)
1-Spring 1982	0.00 (0)	0.00 (0)	0.00 (0)

continued...

TABLE P-47. (CONT'D)

Agent Interval	Low exp level (AEI<1)	Med exp level (1<AEI<5)	High exp level (AEI>5)
Reovirus 1			
0-Baseline	14.22 (17)	12.55 (15)	5.07 (3)
1-Spring 1982	9.58 (3)	14.63 (12)	6.03 (1)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)
3-Spring 1983	0.00 (0)	1.42 (1)	0.00 (0)
4-Summer 1983			
5-1982	5.14 (4)	7.29 (11)	3.15 (1)
6-1983	0.00 (0)	0.89 (1)	0.00 (0)
7-Irrigation	2.99 (3)	5.75 (13)	1.94 (1)
Reovirus 2			
0-Baseline	7.53 (9)	17.57 (21)	11.80 (7)
1-Spring 1982	9.58 (3)	12.19 (10)	0.00 (0)
2-Summer 1982	0.00 (0)	0.00 (0)	0.00 (0)
3-Spring 1983	0.00 (0)	2.83 (2)	0.00 (0)
4-Summer 1983			
5-1982	2.45 (2)	7.12 (11)	0.00 (0)
6-1983	0.00 (0)	1.77 (2)	0.00 (0)
7-Irrigation	2.93 (3)	5.19 (12)	0.00 (0)
Rotavirus			
0-Baseline	0.00 (0)	151.24 (7)	23.50 (4)
1-Spring 1982	0.00 (0)	16.01 (2)	21.56 (1)
2-Summer 1982	19.47 (1)	0.00 (0)	46.3 (3)
3-Spring 1983	0.00 (0)	13.38 (2)	13.66 (1)
4-Summer 1983	0.00 (0)	12.75 (3)	19.97 (3)
5-1982	13.59 (1)	10.62 (3)	24.77 (3)
6-1983	31.06 (1)	19.11 (4)	30.25 (4)
7-Irrigation	8.75 (1)	10.89 (7)	23.91 (8)

a The 95% confidence interval for the high-to-intermediate incidence density ratio does not include the value 1.

b The 90% confidence interval for the high-to-intermediate incidence density ratio does not include the value 1.

c The 95% confidence intervals for both the high-to-low level and high-to-intermediate incidence density ratios do not include the value 1.

d Rates include only nonimmunized participants.

TABLE P-48. DISTRIBUTION OF SEROLOGIC INFECTIONS BY NUMBER OF HOUSEHOLD MEMBERS DONATING SPECIMENS

(Entries are number of households having a specified number of infected members)

Agent	Season[a]	No. with infections per household	No. of household members donating specimens								
			1	2	3	4	5	6	7	8	Total
AD3	0	0	37	40	10	9	6	1	1		104
		1	3	2		1		1			7
		2			2			1			3
AD3	5	0	44	41	11	12	6	3	2	1	120
		1	1	1	1	1	1	2			7
AD5	0	0	38	39	11	9	4	3	1		105
		1	1	3	3						7
AD5	5	0	43	37	12	11	3	7	1	1	115
		1	3		2	1	1	1			8
AD7	0	0	38	39	17	11	7	4	2		118
		1		4			1	1			6
CB2	0	0	37	40	9	6	4	3			99
		1	4	2	2	3			1		12
		2					1				1
CB2	5	0	37	40	11	12	8	3	2		113
		1	3	4		1			1		9
CB4	0	0	34	41	4	6	5	3			93
		1	2	2	6	1		1	1		13
		3							1		1
CB4	2	0	40	42	12	12	4	6	2		118
		1	2	1	1	1					5
CB4	5	0	38	41	10	8	4	5	1		107
		1	4	3	2	4	2		2		17
		2					1				1
CB5	0	0	38	39	16	11	5	3	1		113
		1	1	4	1		2				8
		3							1		1
CB5	1	0	41	41	15	9	6	7	2		121
		1					1		1		2
		2							1		1
CB5	2	0	41	42	13	8	8	6	3	1	122
		1		2	1						3
CB5	4	0	37	33	12	9	7	3	1		102
		1		3			1				4
		2			1				1		2
CB5	5	0	43	38	13	8	6	6	2		116
		1		3			1		1		5
		2								1	1
CB5	6	0	36	33	12	10	6	3	1		101
		1		4			1				5
		2			1				1		2

continued...

TABLE P-48. (CONT'D)

Agent	Season[a]	No. with infections per household	No. of household members donating specimens 1	2	3	4	5	6	7	8	Total
E01	0	0	41	38	17	10	6	4	1		117
		1	2	1		2	1	1			7
E03	0	0	40	41	6	9	9	2			107
		1	3	3	3		1				10
		2		1							1
E03	4	0	39	33	8	10	5	3			98
		1	1	1	2	1	2	2			9
		2					1				1
E03	5	0	45	40	10	11	5	5	1		117
		1		3	1	1	1	3			9
E03	6	0	38	33	8	10	3	3			95
		1	3	2	2	1	3	2			13
		2						1			1
		3						1			1
E09	0	0	42	39	14	10	7	1	2		115
		1	1	2		2					5
		3						1			1
E11	0	0	41	37	14	8	5	3	1		109
		1	3	3	3	1	1	1	1		13
		2				1	1				2
E11	1	0	39	42	16	10	6	4	3		120
		1		1	1	1			1		4
E11	2	0	38	42	15	9	6	3	3		116
		1		2		2	1	1		1	7
E11	4	0	41	34	10	12	3	4	1		105
		1				1	1				2
		2						1	1		2
E11	5	0	39	40	12	7	4	3	3		108
		1	3	3	1	5	2	1	0		15
		2					1		1		2
E11	6	0	38	34	10	11	3	4	1		101
		1		3		1	2				6
		2						1	1		2
E19	5	0	44	39	11	13	6	5	3		121
		1	1	1	1						3
E20	0	0	37	43	13	6	7	4	1		111
		1		3			1		1		5
E20	4	0	35	38	10	8	5	4	2		102
		1	1	1		1					3
		3				1					1
E20	6	0	35	36	10	8	5	4	2		100
		1	1	1	2	1					5
		4					1				1

continued..

TABLE P-48. (CONT'D)

Agent	Season[a]	No. with infections per household	No. of household members donating specimens								
			1	2	3	4	5	6	7	8	Total
E24	0	0	36	40	11	9	8	3	1		108
		1	1	5	1		1				8
E24	4	0	37	38	7	10	6	5			103
		1			1	1					2
		2				1					1
		3							1		1
E24	5	0	41	37	13	12	7	5	2		117
		1	1	6							7
E24	6	0	37	36	7	10	6	5			101
		1		1	2	1					4
		3				1			1		2
RE1	0	0	34	32	10	8	6	1			91
		1	2	7	4	2	1	1	2		19
		2		1	3	1		1			6
		3			1						1
RE1	1	0	37	40	13	9	6	5	4		114
		1	2	6		1	1				10
		2		1		2					3
RE2	0	0	34	30	9	8	3	2	2		88
		1	4	9	6	4	2				25
		2		1		1		2			4
RE2	1	0	37	42	11	9	7	4	3		113
		1		5	2	2	1		1		11
		2				1					1
ROT	0	0	6	5							11
		1	7	2							9
		2			1						1
ROT	1	0	14	10	2	1					27
		1	2	1							3
ROT	2	0	13	12	1	2					28
		1	1		1						2
		2		1							1
ROT	3	0	12	11	1	1					25
		1	1	1		1					3
ROT	4	0	12	7	1	1					21
		1	2	3		1					6
ROT	5	0	14	8	1	2					25
		1	2	2	1						5
		2		1							1
ROT	6	0	12	5	1	1					19
		1	3	4							7
		2				1					1

continued...

TABLE P-48. (CONT'D)

Agent	Season[a]	No. with infections per household	No. of household members donating specimens								
			1	2	3	4	5	6	7	8	Total
LEG	7	0	29	40	7	10	3	2			91
		1	2		1		1				4
		2						1			1
INA	0	0	28	32	6	5	1	1			73
		1	3	6	2	1	3				15
		2		1	1						2
INA	1	0	33	44	11	8	3	3			102
		1	3	1			1	1			6
INA	3	0	33	28	10	3	2	3	2		81
		1	2	5	5		3		1		16
		2		3	1	2	1	1			8
		3				1					1

[a] 0 if baseline period, 1 if spring 1982, 2 if summer 1982, 3 if spring 1983, 4 if summer 1983, 5 if 1982, 6 if 1983, 7 if 1981-1983.

TABLE P-49. DISTRIBUTION OF BACTERIAL INFECTIONS BY NUMBER OF HOUSEHOLD MEMBERS DONATING SPECIMENS

(Entries are number of household having a specified number of infections)

Agent	Season[a]	No. with infections per household	No. of household members donating specimens 1	2	3	4	5	Total
KLB-X[b]	2	0	39	6	4	3		52
		1	3					3
		2		1				1
KLB-W	2	0	39	6	3	2		50
		1	9			1	1	11
		2		1				1
KLB-X	4	0	38	20				58
		1	5	3				8
KLB-W	4	0	38	20				58
		1	7	3				10
		2		1				1
OOB-X	3	0	54	22				76
		1	1	4				5
PBW-X	1	0	59	17	3	2		81
		1	2					2
PBW-W	1	0	59	17	3	2		81
		1	3					3
PBW-X	2	0	45	5	4	2	2	58
		1	3					3
PBW-W	2	0	44	5	4	2	2	57
		1	3	1				4
PBW-X	3	0	51	26				77
		1	2					2
PBW-W	3	0	51	26				77
		1	2					2
PBW-X	4	0	44	25	1			70
PBW-W	4	0	39	21				60
		1	5	4				9

[a] 0 if baseline period, 1 if spring 1982, 2 if summer 1982, 3 if spring 1983, 4 if summer 1983.

[b] X if onset of all infection events during irrigation period, W if includes infection events for which onset may have preceded the irrigation period.

TABLE P-50. APPROXIMATE POWER[a] OF TEST OF THE NULL HYPOTHESIS $p_1=p_2$ AGAINST SPECIFIED ALTERNATIVES OF THE FORM $p_2>p_1$ WITH $\alpha = 0.05$

(The number of individuals in the low exposure group is n_1 and in the high exposure group is n_2. The observed incidence rate in the low exposure group is assumed to be equal to p_1, and the specified alternatives are given by $p_2=p_1 + \Delta$ where Δ = 0.05, 0.07, 0.10, 0.15, 0.20, 0.25. Power less than 0.50 is indicated by a dash.)

				Δ					
Agent	n_1	n_2	p_1	0.05	0.07	0.10	0.15	0.20	0.25
Serologic Agents--Baseline and Control[b]									
AD3	164	91	0.06	-	0.50	0.70	0.90	0.95	0.95
AD5	159	87	0.03	-	0.60	0.80	0.95	0.95	0.95
AD7	198	104	0.01	0.65	0.80	0.90	0.95	0.95	0.95
CB2	156	88	0.06	-	-	0.70	0.90	0.95	0.95
CB4	156	87	0.06	-	-	0.70	0.90	0.95	0.95
CB5	188	94	0.03	-	0.65	0.85	0.95	0.95	0.95
E01	194	97	0.02	0.50	0.70	0.90	0.95	0.95	0.95
E03	164	95	0.05	-	0.50	0.75	0.95	0.95	0.95
E05	168	91	0.01	0.60	0.80	0.90	0.95	0.95	0.95
E09	177	94	0.01	0.55	0.75	0.90	0.95	0.95	0.95
E11	190	95	0.05	-	0.55	0.80	0.95	0.95	0.95
E17	169	97	0.01	0.55	0.75	0.90	0.95	0.95	0.95
E19	171	96	0.01	0.55	0.75	0.90	0.95	0.95	0.95
E20	173	97	0.02	-	0.70	0.85	0.95	0.95	0.95
E24	171	98	0.03	-	0.65	0.85	0.95	0.95	0.95
RE1	186	95	0.16	-	-	0.55	0.85	0.95	0.95
RE2	181	96	0.14	-	-	0.55	0.85	0.95	0.95
ROT	13	17	0.31	-	-	-	-	-	-
INA	132	54	0.11	-	-	-	0.70	0.90	0.95
INA	163	72	0.02	-	0.60	0.80	0.95	0.95	0.95
INA	164	90	0.15	-	-	0.55	0.80	0.95	0.95
LEG	-	-	-	-	-	-	-	-	-
POR	133	82	0.04	-	0.50	0.75	0.90	0.95	0.95
WWV	-	-	-	-	-	-	-	-	-
SNV	138	70	0.45	-	-	-	0.60	0.80	0.95
Serologic Agents--Spring 1982									
AD3	185	106	0.00	0.75	0.85	0.95	0.95	0.95	0.95
AD5	186	101	0.02	0.55	0.75	0.90	0.95	0.95	0.95
AD7	198	108	0.00	0.75	0.90	0.95	0.95	0.95	0.95
CB2	190	104	0.00	0.75	0.85	0.95	0.95	0.95	0.95
CB4	188	108	0.01	0.70	0.85	0.95	0.95	0.95	0.95
CB5	197	110	0.01	0.65	0.80	0.95	0.95	0.95	0.95
E01	197	110	0.01	0.70	0.85	0.95	0.95	0.95	0.95
E03	187	101	0.00	0.75	0.85	0.95	0.95	0.95	0.95
E05	189	103	0.01	0.65	0.85	0.95	0.95	0.95	0.95
E09	193	108	0.00	0.75	0.90	0.95	0.95	0.95	0.95

continued...

TABLE P-50. (CONT'D)

Agent	n_1	n_2	p_1	Δ					
				0.05	0.07	0.10	0.15	0.20	0.25
Serologic Agents--Spring 1982 (Cont'd)									
E11	199	104	0.01	0.65	0.80	0.90	0.95	0.95	0.95
E17	190	106	0.01	0.70	0.85	0.95	0.95	0.95	0.95
E19	186	103	0.00	0.75	0.85	0.95	0.95	0.95	0.95
E20	191	104	0.01	0.65	0.85	0.95	0.95	0.95	0.95
E24	182	105	0.01	0.65	0.80	0.95	0.95	0.95	0.95
RE1	202	111	0.05	–	0.60	0.80	0.95	0.95	0.95
RE2	200	110	0.05	–	0.60	0.80	0.95	0.95	0.95
ROT	24	24	0.04	–	–	–	–	–	0.60
LEG	148	65	0.03	–	0.55	0.75	0.90	0.95	0.95
POR	124	64	0.07	–	–	0.55	0.80	0.95	0.95
WWV	146	76	0.05	–	–	0.65	0.90	0.95	0.95
SNV	122	61	0.10	–	–	–	0.75	0.90	0.95
Serologic Agents--Summer 1982									
AD3	231	69	0.00	0.65	0.80	0.90	0.95	0.95	0.95
AD5	228	66	0.01	0.55	0.75	0.90	0.95	0.95	0.95
AD7	222	55	0.00	0.65	0.75	0.90	0.95	0.95	0.95
CB2	224	65	0.00	0.60	0.75	0.90	0.95	0.95	0.95
CB4	223	66	0.01	0.50	0.85	0.70	0.85	0.95	0.95
CB5	237	71	0.01	0.60	0.75	0.90	0.95	0.95	0.95
E01	223	56	0.00	0.65	0.80	0.90	0.95	0.95	0.95
E03	229	69	0.01	0.55	0.70	0.85	0.95	0.95	0.95
E05	222	54	0.00	0.65	0.75	0.90	0.95	0.95	0.95
E09	219	54	0.00	0.65	0.75	0.90	0.95	0.95	0.95
E11	235	68	0.02	–	0.65	0.85	0.95	0.95	0.95
E17	234	70	0.00	0.70	0.85	0.90	0.95	0.95	0.95
E19	230	68	0.00	0.70	0.80	0.90	0.95	0.95	0.95
E20	234	67	0.00	0.65	0.80	0.90	0.95	0.95	0.95
E24	226	70	0.00	0.70	0.80	0.90	0.95	0.95	0.95
RE1	–	–	–	–	–	–	–	–	–
RE2	–	–	–	–	—	–	–	–	–
ROT	36	18	0.03	–	–	–	–	0.55	0.65
LEG	–	–	–	–	–	–	–	–	–
POR	164	44	0.05	–	–	0.55	0.80	0.90	0.95
WWV	193	57	0.05	–	–	0.65	0.85	0.95	0.95
SNV	150	40	0.11	–	–	–	0.65	0.80	0.90
Serologic Agents--Spring 1983									
AD3	175	97	0.01	0.65	0.80	0.90	0.95	0.95	0.95
AD5	173	93	0.01	0.55	0.75	0.90	0.95	0.95	0.95
AD7	178	99	0.00	0.70	0.85	0.95	0.95	0.95	0.95
CB2	–	–	–	–	–	–	–	–	–

continued...

TABLE P-50. (CONT'D)

Agent	n_1	n_2	p_1	Δ					
				0.05	0.07	0.10	0.15	0.20	0.25
Serologic Agents--Spring 1983 (Cont'd)									
CB4	-	-	-	-	-	-	-	-	-
CB5	174	100	0.01	0.65	0.80	0.90	0.95	0.95	0.95
E01	177	102	0.00	0.70	0.85	0.95	0.95	0.95	0.95
E03	175	93	0.02	0.50	0.70	0.90	0.95	0.95	0.95
E05	177	99	0.00	0.70	0.85	0.95	0.95	0.95	0.95
E09	175	98	0.01	0.65	0.80	0.90	0.95	0.95	0.95
E11	178	97	0.00	0.70	0.85	0.95	0.95	0.95	0.95
E17	172	97	0.00	0.70	0.85	0.95	0.95	0.95	0.95
E19	172	95	0.00	0.70	0.85	0.95	0.95	0.95	0.95
E20	167	98	0.00	0.70	0.85	0.95	0.95	0.95	0.95
E24	171	98	0.01	0.55	0.75	0.90	0.95	0.95	0.95
RE1	159	90	0.00	0.65	0.80	0.90	0.95	0.95	0.95
RE2	159	90	0.01	0.60	0.75	0.90	0.95	0.95	0.95
ROT	21	27	0.05	-	-	-	-	-	0.50
LEG	-	-	-	-	-	-	-	-	-
POR	-	-	-	-	-	-	-	-	-
WWV	-	-	-	-	-	-	-	-	-
SNV	137	75	0.07	-	-	0.60	0.85	0.95	0.95
Serologic Agents--Summer 1983									
AD3	197	59	0.00	0.65	0.75	0.90	0.95	0.95	0.95
AD5	191	57	0.01	0.55	0.70	0.85	0.95	0.95	0.95
AD7	196	61	0.00	0.65	0.80	0.90	0.95	0.95	0.95
CB2	-	-	-	-	-	-	-	-	-
CB4	-	-	-	-	-	-	-	-	-
CB5	197	59	0.04	-	0.50	0.70	0.90	0.95	0.95
E01	197	61	0.00	0.65	0.80	0.90	0.95	0.95	0.95
E03	194	58	0.04	-	0.50	0.70	0.90	0.95	0.95
E05	197	59	0.00	0.65	0.75	0.90	0.95	0.95	0.95
E09	196	59	0.01	0.55	0.70	0.85	0.95	0.95	0.95
E11	196	59	0.02	-	0.65	0.80	0.95	0.95	0.95
E17	193	58	0.00	0.60	0.75	0.90	0.95	0.95	0.95
E19	194	57	0.00	0.60	0.75	0.90	0.95	0.95	0.95
E20	192	55	0.02	-	0.55	0.75	0.90	0.95	0.95
E24	193	58	0.02	-	0.65	0.80	0.95	0.95	0.95
RE1	-	-	-	-	-	-	-	-	-
RE2	-	-	-	-	-	-	-	-	-
ROT	24	21	0.13	-	-	-	-	-	-
LEG	-	-	-	-	-	-	-	-	-
POR	-	-	-	-	-	-	-	-	-
WWV	-	-	-	-	-	-	-	-	-
SNV	160	49	0.14	-	-	-	0.65	0.85	0.95

continued..

TABLE P-50. (CONT'D)

				Δ					
Agent	n_1	n_2	p_1	0.05	0.07	0.10	0.15	0.20	0.25
Fecal Agents--Spring 1982									
KLB-X	68	42	0.00	–	–	0.60	0.80	0.90	0.95
KLB-W	70	42	0.03	–	–	–	0.70	0.85	0.95
OOB-X	71	42	0.00	–	–	0.65	0.80	0.90	0.95
OOB-W	71	42	0.00	–	–	0.65	0.80	0.90	0.95
PBW-X	70	42	0.01	–	–	0.55	0.75	0.90	0.95
PBW-W	71	42	0.03	–	–	–	0.70	0.85	0.95
VIR-X	72	42	0.08	–	–	–	0.60	0.75	0.90
VIR-W	77	43	0.14	–	–	–	0.50	0.70	0.85
WWI-X	65	40	0.06	–	–	–	0.60	0.80	0.90
WWI-W	69	41	0.12	–	–	–	0.50	0.70	0.85
Fecal Agents--Summer 1982									
KLB-X	59	21	0.05	–	–	–	–	0.60	0.75
KLB-W	65	23	0.14	–	–	–	–	0.50	0.70
OOB-X	65	23	0.00	–	–	0.50	0.70	0.80	0.90
OOB-W	66	24	0.02	–	–	–	0.60	0.75	0.85
PBW-X	65	23	0.03	–	–	–	0.55	0.70	0.85
PBW-W	65	24	0.03	–	–	–	0.55	0.75	0.85
VIR-X	79	26	0.08	–	–	–	0.50	0.70	0.80
VIR-W	80	26	0.09	–	–	–	–	0.65	0.80
WWI-X	59	19	0.14	–	–	–	–	–	0.60
WWI-W	64	22	0.20	–	–	–	–	–	0.60
Fecal Agents—Spring 1983									
KLB	60	47	0.00	–	–	0.60	0.80	0.90	0.95
OOB	60	47	0.03	–	–	–	0.70	0.85	0.95
PBW	60	45	0.03	–	–	–	0.70	0.85	0.95
VIR	62	47	0.00	–	–	0.60	0.80	0.90	0.95
WWI	59	45	0.03	–	–	–	0.65	0.85	0.90
Fecal Agents—Summer 1983									
KLB-X	65	24	0.05	–	–	–	0.50	0.70	0.80
KLB-W	67	26	0.07	–	–	–	–	0.65	0.80
OOB-X	67	26	0.01	–	–	–	0.65	0.80	0.90
OOB-W	68	26	0.03	–	–	–	0.60	0.75	0.85
PBW-X	62	23	0.00	–	–	–	0.65	0.80	0.90

continued...

TABLE P-50. (CONT'D)

				Δ					
Agent	n1	n2	p1	0.05	0.07	0.10	0.15	0.20	0.25
Fecal Agents--Summer 1983 (Cont'd)									
PBW-W	68	26	0.09	-	-	-	-	0.65	0.80
VIR-X	69	25	0.01	-	-	-	0.65	0.80	0.90
VIR-W	72	25	0.06	-	-	-	0.50	0.70	0.80
WWI-X	60	21	0.05	-	-	-	-	0.65	0.75
WWI-W	69	26	0.17	-	-	-	-	0.50	0.70

a Approximate power calculations use the method of Fleiss et al. (1980).

b See Table 112 for exact periods of observation.

GLOSSARY

Study Objective

The general objective of the LISS was to identify possible adverse effects on human health from slow rate (sprinkler) land application of wastewater which contained potentially pathogenic microorganisms. More precisely, the objective was to determine the association, if any, between the occurrence of infectious diseases in residents and workers and their exposure to the wastewater and aerosols produced by wastewater spray irrigation. This objective was accomplished by disease surveillance of the study population, by description of the distribution of infections, and principally by evaluation of the incidence of infections for association with exposure.

Disease Surveillance

Disease surveillance was the continuing scrutiny of all aspects of occurrence and spread of infectious diseases in the study population. Included were the systematic collection and evaluation of self-reported illness information, investigation of cases and outbreaks for source of illness, isolation and identification of infectious agents from routine and illness specimens, testing sequential blood samples for evidence of infection, and other relevant epidemiological data. The primary function of this activity was the protection of the population from any obvious untoward effects.

Illness Prevalence Density

The illness prevalence density was defined as the number of person-days of self-reported illness per 1000 person-days of observation.

Illness Incidence Density

The illness incidence density was defined as the number of new illnesses reported per 1000 person-days of observation.

Bacterial Infection

A fecal donor was considered to be having a bacterial infection when an overt or opportunistic bacterial pathogen was isolated from a fecal specimen at or exceeding a specified semiquantitative level which might be associated with enteric disease. The levels equated with bacterial infection were:

Category 1	any isolate of a major enteric bacterial pathogen (i.e., Salmonella or Shigella species, Campylobacter jejuni, or Yersinia enterocolitica);

Category 2	isolation at the heavy level of a possibly significant opportunistic pathogen (i.e., API Group I, *Candida albicans*, *Chromobacterium*, *Citrobacter*, *Klebsiella*, *Morganella*, *Proteus*, *Providencia*, *Serratia*, and *Staphylococcus aureus*);
Category 3	isolation at the moderate or heavy level of selected organisms found to be uncommon in feces but prominent in the sprayed wastewater (i.e., *Aeromonas hydrophila* and the fluorescent *Pseudomonas* group: *P. aeruginosa*, *P. fluorescens*, and *P. putida*).

Bacterial Infection Event

A bacterially infected fecal donor was considered to have had a bacterial infection event since donation of the prior fecal specimen in the series when the level of the organism in the prior specimen had been:

1) negative, for major enteric pathogens,
2) negative to light, for possibly significant opportunistic pathogens,
3) negative to light, for organisms prominent in the wastewater.

The criteria for a bacterial infection event were summarized for all three bacterial pathogen categories in Table 10.

It was of primary interest to determine the bacterial infection status of a routine fecal specimen donor in relation to a period of irrigation. Routine specimens were collected from designated donors in scheduled weeks before, during and near the end of each irrigation period (see Figure 2), usually at intervals of about 6 and 4 weeks, respectively. Thus, the onsets of bacterial infection events could be temporally related to wastewater irrigation periods. When the change in infection status occurred between the two specimens donated during an irrigation period, onset occurred in the interim (i.e., during the irrigation period). When the change in infection status occurred in consecutive specimens donated before and during the irrigation period, it was uncertain whether onset occurred after irrigation commenced. When a bacterial agent was not recovered at a level equated with infection in either routine fecal specimen provided during an irrigation period, the donor was considered to have experienced no infection events by the agent during the observation period preceding and spanning the collection dates of the consecutive specimens.

Viral Infection Event

A viral infection event was defined as the detection of a specific virus by laboratory cultivation or by EM examination in the second and not the first of paired fecal specimens from the same person. Subsequent recovery of the same virus in a specimen from the same individual would be a new event if more than 6 weeks elapsed between sequential recoveries. Detection of a virus in the first of serial specimens was also considered a viral infection event. Viral infection status was correlated with an irrigation period in the same manner as bacterial infection status.

Serological Antibody Titer

The serological antibody titer was the reciprocal of the highest serum dilution at which a predefined endpoint of reaction was observed.

Serological Infection Event (Serological Conversion)

A serological conversion (''seroconversion'') was defined as a fourfold or greater rise in agent-specific antibody titer in successive sera from one individual that were tested simultaneously. Since successive sera from 1982 and 1983 spanned an irrigation period and several additional months (see Figure 2), it was not possible to determine if the onset of serologically detected infection events was during the irrigation period.

Serological Infection Incidence Density (Seroconversion Incidence Density)

The serological infection incidence density was defined as the number of serological infection events per hundred person-years of observation. ID was calculated as:

$$\text{ID} = \frac{\text{Number of Serological Infection Events in Time Interval}}{\text{Number of Person-days Observed During Interval}} \times (365.25\ \text{days/yr}) \times (100\ \text{yr})$$

Infection Episode

An infection episode was defined as the observation in the study population of a number of similar infection events (either serologically, microbiologically, or clinically) within a restricted interval of time. The minimum number of infections which constituted an infection episode was set by determining the number of infections that would be needed to reject the null hypothesis (of no association between infection status and wastewater exposure), assuming that all of the infections occurred in the high exposure group and no infections occurred in the low exposure group. Infection episodes were classified as exposure situations when the observation period corresponded to one or two major irrigation periods and when the causative agent was found (or could be presumed) to be present in the wastewater at that time. Infection episodes were classified as control situations when the causative agent could not survive in wastewater (i.e., influenza A) or when the episode preceded the start of irrigation. Each exposure and control infection episode was statistically analyzed for association with wastewater aerosol exposure.